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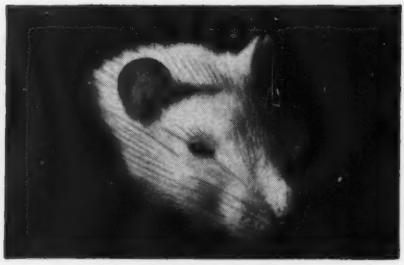
WHY



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[1] Harrington, C. R.: "The Thyroid Gland," Oxford, 1933, p. 141.
[2] Meyer, A. E., and Wertz, A.: Endocrinology 24: 806, 1939.

PROLOID the improved thyroid

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THE PROTEOLYTIC ACTIVITY OF NORMAL AND PATHOLOGICAL HUMAN THYROID TISSUE[1]

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THE importance of proteolytic enzyme systems in the synthesis of the thyroid hormone has been recognized since Salter and Pearson (25), using pepsin, were able to synthesize iodoproteins possessing activity similar to that of thyroglobulin. The same results were obtained by Salter and Lerman (24) with extracts of whole thyroid glands. These authors suggested the existence of an enzyme system which could either synthesize or hydrolyse the proteins according to the thermodynamic conditions. Gersh and Caspersson (7) suggested the possibility that enzymes in the thyroid gland hydrolyse thyroglobulin into polypeptides or peptones which could pass the cell membranes.

In 1941 one of us (3) was able to demonstrate the existence of definite proteolytic activity in the colloid extracted by microdissection from a single follicle, and to establish a relation between this activity and the function of the thyroid gland. From these results, which were afterwards confirmed for the total gland by Dziemian (5), it was concluded that in the reabsorption of the follicular colloid an enzyme system may be involved, which is responsible for the hydrolysis of thyroglobulin and its breakdown into smaller molecules, and that the rate of this hydrolysis may depend upon the state of activity of the thyroid.

These investigations strongly support the assumption that a similar

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[1] This work was aided by a grant made to the Institute of General Histology and Embryology by the Rockefeller Foundation.

mechanism exists in the human gland and that changes in this enzymatic activity may be related to disease of the thyroid. In the present paper we undertake to investigate this hypothesis and to carry out estimations of the enzymatic activity of a series of human thyroid glands representing the normal and various pathological conditions. The results have fully confirmed the assumption, demonstrating an important role of the postulated enzyme system in the physiopathology of thyrotoxicosis and simple thyroid goiter. Our results also suggest indirectly that the therapeutic action of iodine in thyrotoxicosis may be attributed to the direct influence of iodine upon the proteolytic enzyme system of the thyroid.

METHODS

Thirty-nine normal and pathological thyroid glands were investigated. The material was obtained during surgical operations, and in each case all the clinical data of interest for our problem were available, as well as information obtained from physical and laboratory examinations. Immediately after removal pieces of the gland were frozen in liquid air and kept in an icebox at −35°C. At this temperature proteolytic activity is maintained at a constant level for at least one year.

The extraction of the enzyme was done according to the method described by Dziemian (5). Pieces of the gland were finely minced, weighed and extracted for 22 hours with 60 per cent glycerol in the proportion of 0.5 cc. of glycerol to 100 mg. of tissue. Two hours before the experiment the extract was diluted 1:1 with distilled water.

The degree of proteolysis was measured with the Pulfrich photometer, by determining the amount of liberated tyrosine and tryptophane. For the estimation of these amino acids we used the method described by Folin and Ciocalteu (6), adapting it to the photometric conditions because the blue color developed in this reaction follows the Lambert-Beer law. The calibration curve was prepared with appropriate quantities of tyrosine. Therefore all the figures cited in this paper give the amounts of liberated tyrosine and tryptophane, and are expressed in milligram-equivalents of tyrosine. The separate estimation of these two amino acids seems to us not to be essential for our purpose, particularly because their relative proportions are reasonably constant. Each digest contained 0.25 cc. of a four per cent solution of edestin[2] at pH 4.0 as substrate; 0.25 cc. of citrate buffer at pH 4.0; 0.25 cc. of distilled water and 0.25 cc. of a glycerol extract of the gland. Particular care was taken to maintain the digest at pH 4.0 throughout the experiment. Incubation was carried on for four hours at

[2] The edestin was kindly prepared by Dr. Walter Fraenkel, formerly of the firm, Dr. Fraenkel and Dr. Landau, of Berlin.

38°C. Proteolysis was stopped by the addition of 1 cc. of a 3 per cent solution of trichloracetic acid, and the precipitate was filtered off through a Buchner funnel using Whatman filter paper No. 42.

The precipitate was washed with 10 cc. of distilled water which was added to the filtrate. The filtrate was then transferred into a 25 cc. measuring flask and 8 cc. of a concentrated solution of sodium carbonate and 1 cc. of the phenol reagent were added. The contents were then brought up to the mark with distilled water. The readings were carried out after 15 minutes, using the optical filter No. S79. For each experiment three tubes were run with the same digest. The figures given herewith are an average of the values obtained from the three separate estimations.

Samples of the glands were also taken for histological and cytological control; they were dried in the Altmann-Gersh apparatus at −30°C, embedded in nitrocellulose, and cut and stained as described by De Robertis (3).

RESULTS

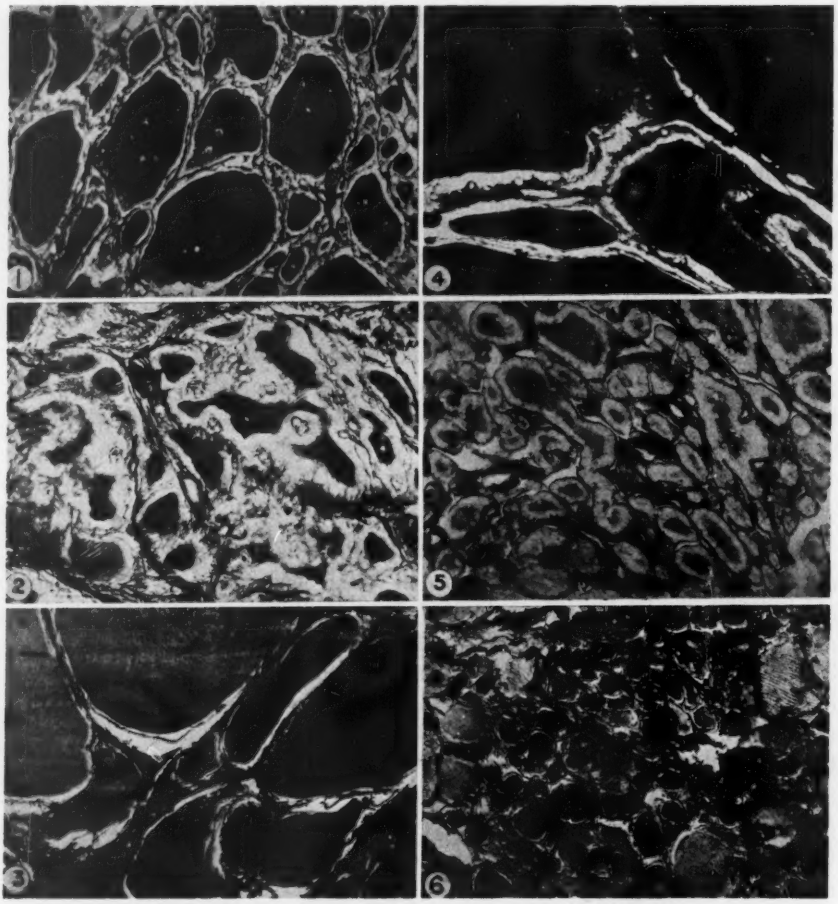
A. Normal thyroid

Because glands of completely normal individuals were very difficult to obtain we considered as normal those in which there was a localized lesion, obtained from patients who did not show clinical symptoms of disease, nor any change of basal metabolic rate (BMR). Specimens No. 356, 357, 382, 414 and 638 were obtained from cases in which there was a simple encapsulated adenoma. The samples were taken as far as possible from the tumor and were of completely normal appearance. Those cases which were histologically investigated showed normal structure of the gland (Figure 1). Specimens 399, 413b, and 637 were obtained from cases of localized cystic goiter. *Case 387* was a patient having carcinoma of the larynx and the thyroid had a completely normal appearance; *Case 522* was a patient with carcinoma of the pharynx.

As seen from Table 1, in all these cases the proteolytic activity, as measured by the tyrosine and tryptophane liberated by digestion of an extract obtained from 100 mg. of thyroid tissue, varied between 0.37 mg. and 0.53 mg., the average being 0.444 mg. The scattering observed was less than that observed by Dziemian (5) in normal thyroid glands of the rat.

B. Severe diffuse toxic goiter

The specimens described under this heading (Table 1) came from cases of toxic goiter in which the whole gland was enlarged and which exhibited very severe symptoms of thyrotoxicosis. In all these cases the basal metabolic rate before the treatment with iodine was above +40 per cent and at the time of the operation had not been brought down to the normal



FIGS. 1–6. Human thyroid glands, frozen and dried *in vacuo* and stained with aniline blue-orange green (Obj., 8 mm.; Homal, II; camera length, 50 cm.

FIG. 1. Normal gland, *Case 382*. Proteolytic activity: 0.39.

FIG. 2. Severe toxic goiter, *Case 434*. Proteolytic activity: 1.07.

FIG. 3. Mild toxic goiter, *Case 386*. Proteolytic activity: 0.24.

FIG. 4. Mild diffuse goiter (colloid), *Case 372*. Proteolytic activity: 0.20.

FIG. 5. Toxic nodular goiter, *Case 412*, portion from the adenoma. Proteolytic activity: 0.92.

FIG. 6. Same case as Fig. 5, portion of the rest of the gland. Proteolytic activity: 0.53.

level, being in general above +15 per cent. An exception was in *Case 493*, a toxic goiter of five months' duration, treated for two months with diiodotyrosine; the gland was but slightly enlarged and the basal metabolic rate was +75 per cent; shortly before the operation the basal metabolic rate dropped to +5 per cent.

In *Case 354* the basal metabolic rate also reached a low level (+9 per cent), but this was because of previous x-ray therapy. The patient exhibited typical signs of exophthalmic goiter, with a basal metabolic rate of

TABLE 1

Normal thyroids		Severe diffuse toxic goiter				Mild toxic goiter				Simple diffuse goiter	
Case no.	Proteolytic activity per 100 mg. tissue	Case no.	Proteolytic activity per 100 mg. tissue	Initial BMR per cent	BMR before operation per cent	Case no.	Proteolytic activity per 100 mg. tissue	Initial BMR per cent	BMR before operation per cent	Case no.	Proteolytic activity per 100 mg. tissue
356	0.39	325	0.63	+ 45	+27	352	0.35	+23	—	372	0.22
357	0.53	354	0.85	+ 65	+ 9	355	0.24	+19	+7	435	0.43
382	0.52	388	0.75	+ 84	+24	374	0.38	+40	−0.5	436	0.32
387	0.43	407	1.12	+ 80	+57	386	0.26	+27	+6	494	0.25
399	0.37	434	1.07	+100	+35	437	0.28	+35	+4	499	0.34
413	0.47	438	0.69	+ 94	+26	439	0.41	+40	+7	523	0.27
414	0.46	493	0.98	+ 75	+ 5	615	0.42	+20	−6	525	0.45
522	0.42	495	0.74	+124	+39					636	0.30
637	0.47	497	0.80	+ 40	+15						
638	0.40	526	1.27	+ 66	+33						
		626	0.72	—	+43						
Ave.	0.444		0.872				0.332				0.320

+65 per cent, and treatment with Lugol's solution was unsuccessful; hence the radiation therapy.

In *Case 626* the thyrotoxicosis was extremely severe. The initial basal metabolic rate was unknown, and treatment with iodine produced little symptomatic improvement; three days before the operation the basal metabolic rate was +43 per cent.

As shown in Table 1, in all these cases the proteolytic activity was much higher than in the normal case, *i.e.*, between 0.63 mg. and 1.27 mg. The average figure was 0.872 mg. or about 96 per cent above the normal level. It is clear therefore that in cases of severe toxic goiter the enzymatic

activity is greatly increased, although to a variable degree. It is impossible to correlate in every case the degree of increase of enzymatic activity with the severity of the symptoms or with the basal metabolic rate, but on the whole they are parallel. *Case 497*, which because of the relatively low initial basal metabolic rate (+40 per cent) could be put in the next group, after 20 days of treatment, showed resistance to iodine.

C. Mild toxic goiter

Under this heading are grouped seven cases of diffuse goiter, which have in common the mildness of the toxic symptoms (basal metabolic rate below +40 per cent) and a complete normalization of the basal metabolic rate during the preoperative administration of iodine. The proteolytic activity of this group lay between 0.24 mg. and 0.42 mg., the average of all the cases being 0.332 mg. This value is about 26 per cent below normal rate. These figures seem to indicate that when the toxic symptoms of a mild case are completely relieved by the iodine treatment, the proteolytic activity, which before treatment was presumably above normal, decreases to a level below that of the normal gland. It is interesting to note that the cases studied histologically showed the appearance of colloid goiter (Figure 3).

D. Simple diffuse goiter

The eight specimens of this group came from cases of diffuse goiter with no symptoms of thyrotoxicosis. In general, the glands were greatly increased in size after slow growth for years. *Cases 499* and *525*, with a history of nine and 21 years respectively, are patients who came from regions where goiter is endemic. The basal metabolic rate on the whole was in the normal range, but in some cases slightly lower (in *Case 372* it was −11 per cent; in *Case 435*, −8 per cent). No general symptoms were observed.

The proteolytic activity varied between 0.22 mg. and 0.45 mg. with an average of 0.32 mg., or about 30 per cent of that of the normal gland. Histological control showed typical appearances of colloid goiter (Figure 4).

E. Nodular toxic goiter

We had at our disposal only two cases in which the tissue could be classified as nodular toxic goiters of Plummer's type (toxic adenoma). In *Case 412* the patient had had for ten years a nodule in the right lobe of the gland, but only in the last three years were there any symptoms of thyrotoxicosis. The basal metabolic rate was +35 per cent one month before the operation. An encapsulated nodule the size of a hen's egg was removed during the operation. The proteolytic activity of the nodule was completely different from that of the surrounding tissue (0.93 and 0.53

mg. respectively). Histological comparison of these two parts of the gland also showed remarkable differences (Figures 5 and 6).

In *Case 433* the patient had symptoms of a severe thyrotoxicosis two years before the operation, the basal metabolic rate oscillating between +66 per cent and +25 per cent. At operation an encapsulated adenoma was found. The proteolytic activity of this adenoma and of the surrounding tissue, as in the former case, also gave different figures (0.97 mg. and 0.43 mg.).

No final conclusions can be drawn from these two cases, but it is interesting to note that there was a significant difference between the proteolytic activity of the adenoma and that of the rest of the gland.

DISCUSSION

A. Proteolytic activity of the normal gland and of toxic goiter

The present results show that in the human thyroid gland proteolytic activity exists, as has been previously demonstrated in the rat by De Robertis (3) and Dziemian (5) and in the guinea pig by Dziemian (5). The fact that the optimum activity occurs at pH 4.0 seems to confirm Dziemian's supposition that this enzyme is of a catheptic type. Our own and also Dziemian's experimental results have shown that the catheptic activity is increased in the colloid (3) and in the whole gland activated under the influence of the thyrotrophic hormone (5). In the rat it reaches 30 per cent above that of the normal gland. This increase is possibly correlated with the release of larger quantities of hormone. The similar increase of proteolytic activity seen in thyrotoxicosis, which reaches about 96 per cent above the normal level (Table 1) may also reflect a higher output of the thyroid hormone. The interpretation of our results is rather difficult because other factors, above all preoperative iodine therapy, influence the proteolytic activity (see below).

All the patients having toxic goiter presented in Table 1, with the exception of that of *Case 493*, were treated with iodine, and all of them showed at the time of operation an improvement of toxic symptoms, without reaching normality. We may therefore assume that without the iodine treatment our results might possibly be much higher and might then be better correlated with the clinical symptoms.

Our results support the hypothesis of an enzyme system controlling reabsorption of the colloid, which postulates that thyroglobulin, being a large molecule of the molecular weight of 675,000 (11, 12) cannot, as such, pass through the cell membrane. It must therefore be broken down into smaller molecules by the action of a proteolytic enzyme (3). Indirect support of this theory is provided by the work of Lerman (17), who could not demonstrate by immunological methods the presence of thyroglobulin

in the circulating blood even in cases of thyrotoxicosis, and by the work of Riggs, Lavietes, and Man (23), who showed that the organic iodine-containing fraction of the blood can be considered to have a molecular weight of 69,000 or even less.

The theory that in thyrotoxicosis there is an increased "leakage" of the gland which causes depletion of the colloidal stores is supported by many authors, and particularly by Holst (14) and Harington (10). This "leakage" may be explained on a biochemical basis without looking for purely mechanical interpretations, by the great increase of proteolytic activity which we have shown to occur in cases of severe thyrotoxicosis.

The results obtained in the two cases of nodular toxic goiter are in accordance with this biochemical concept. They show a great difference between the proteolytic activity of the adenoma and of the rest of the gland which is probably related to the different outputs of hormone in these two regions of the thyroid. These differences are also manifest histologically, as all the signs of increased activity (including colloid depletion) are present in the adenoma, whereas in the rest of the gland the follicles are small, but have a low epithelium and are completely filled with colloid (Figures 5 and 6).

B. Proteolytic activity of simple goiter

All the cases of simple goiter presented in Table 1 had a long evolution so that they reached the colloid stage of Marine's classification. Histologically they showed large follicles with atrophic epithelium, distended by colloid (Figure 4). Physiologically, these glands may be considered as hypoactive, even though the patients were in the euthyroid state, because the output of the hormone per unit weight of tissue was greatly diminished. Even in the blood there was generally a lower iodine concentration and therefore a tendency toward a hypothyroid state of the organism. With decreasing release of the hormone, however, there was a significant diminution of the proteolytic activity (about 30 per cent). It is interesting to note in this connection that in hypophysectomized rats, in which the release of the colloid is reduced, Dziemian (5) found a decreased proteolytic activity (22.3 per cent below the normal).

These facts strongly support the hypothesis that in the development of colloid goiter the proteolytic enzyme system is involved, and this in turn affects the enzymatic reabsorption of the colloid.

C. Proteolytic activity and the mechanism of the therapeutic action of iodine

Since the introduction of the iodine treatment of toxic goiter by Plummer (22) various authors have tried to explain the mechanism of its action

on the thyroid gland. This action is characterized clinically by the relief of symptoms, including a drop in basal metabolic rate (20); physiologically, by the apparent decrease of the circulating hormone (9, 15, 16) and increase of total iodine in the thyroid (8, 18); and histologically, by deposition of colloid within the follicles (1). In order to explain all these facts it is generally agreed that iodine acts by blocking the release of the secretion from the thyroid follicles, but the actual mechanism is still not satisfactorily explained. Marine (19) thought that storage of colloid induced by iodine interferes with delivery of the colloid into the circulation, and Mosser (21) suggested that the stored colloid compresses the cells and decreases their secretory activity. These hypotheses of mechanical blockage are no longer necessary in view of what we know of the histophysiology and the enzyme chemistry of the thyroid gland.

Salter and Lerman (24) from studies of enzymatic synthesis carried out with proteases as catalysts, concluded that the therapeutic effect of iodine is caused by a mass-law phenomenon which "forces" the reaction in the direction of synthesis, inducing formation and storage of colloid.

In 1941 one of us [De Robertis (3)] demonstrated that after treatment with iodine for a long period the colloid extracted from a single follicle of the rat thyroid shows a decrease in proteolytic activity. This result was afterwards confirmed by Dziemian (5) in the whole gland.

The results obtained in cases of mild thyrotoxicosis, together with the above mentioned results on rats, fully support the idea that iodine acts on the thyroid by inhibiting the proteolytic enzyme system. In fact, the seven cases of mild thyrotoxicosis which had a complete relief of symptoms, including normalization of the basal metabolic rate, as the result of iodine therapy, showed a proteolytic activity not only lower than that found in severe toxic goiter, but even below the level of that of normal glands (-26.2 per cent). Histologically these cases showed large follicles filled with colloid, resembling colloid goiter. It is interesting to point out that colloid goiters also showed a level of proteolytic activity very closely resembling that found in mild thyrotoxic cases (-27.9 per cent).

On the basis of all the foregoing considerations we suggest that iodine inhibits the proteolytic enzyme system responsible for hydrolysis of the thyroglobulin, thus reducing the release of colloid and favoring its storage within the follicle. The mechanism of iodine action in this inhibition may possibly (it seems to us) be due to a direct iodinization of the proteolytic enzyme. Herriott (13) observed a progressive inhibition of the activity of pepsin iodinized *in vitro*. This inhibition depends upon the fixation of iodine in the tyrosine molecule by the formation of diiodotyrosine.

In order to check the direct action of iodine upon the proteolytic enzyme of the thyroid, we carried out a series of experiments in which the glycerol

extracts of the thyroid (toxic goiter) were iodinized *in vitro* by Herriott's method. The results thus obtained showed a strong inhibition of the proteolytic activity (4). It should be stressed that only free iodine has such an effect on the proteolysis, for with KI only no change in the activity could be observed. Similar negative results were obtained with thiourea.

Our iodinization hypothesis does not exclude the existence of other inhibitory mechanisms, such as the mass-law action suggested by Salter and Lerman (24). It is obvious that in the case of a process so important physiologically as the regulation of hormone production, the cooperation of several systems must be involved.

SUMMARY

The rate of proteolytic activity of normal and pathological human thyroid tissue was measured photometrically by the estimation of tyrosine and tryptophane liberated from edestin as substrate, using the phenol reagent of Folin-Ciocalteu. Under arbitrarily fixed conditions the proteolytic activity of the normal gland yielded from 0.37 mg. to 0.57 mg. of tyrosine and tryptophane (expressed as mg. equivalents of tyrosine) per 100 mg. of tissue. Under the same conditions, in severe toxic goiters the proteolytic activity was found to be about 96 per cent above that of the normal gland. In mild toxic goiters, in which the symptoms, including the elevated basal metabolic rate, were normalized by treatment with iodine, the proteolytic activity was decreased to about 26 per cent below the normal level. Simple diffuse colloid goiters showed a decrease of proteolytic activity corresponding to 27.9 per cent below normal. In two cases of nodular toxic goiter (toxic adenoma) a difference of almost 100 per cent was found between the proteolytic activity of the adenoma and that of the surrounding tissue. The authors conclude that the proteolytic enzyme system plays an important role in the release of colloids and in the physiopathology of toxic and simple goiters. They also conclude that the therapeutic effect of iodine is probably partly due to inhibition of the proteolytic system responsible for the breakdown of the thyroglobulin molecule, thus preventing release of the hormones from the follicle. It is assumed that the mechanism of this inhibition involves direct iodinization of the proteolytic enzyme inside the thyroid gland.

ACKNOWLEDGMENTS

We wish to express our thanks to Prof. Dr. O. Ivanissevich, Director of the Instituto de Cirurgía, Faculdad de Medecina, Buenos Aires, for facilities in obtaining most of the material, and to Drs. Rey, Ferarri, Luchetti, Bengolea, and Profs. Egües and Del Castillo, for supplying us with extirpated goiters. Thanks are also due to Prof. Dr. A. B. Marenzi for suggestions as to methods and for supplying us with tyrosine for the photo-

metric calibration studies. We wish to express our gratitude to Prof. Dr. Manuel E. Varela, Director of this Institute, for his continued interest in our work, and also to Dr. George W. Corner, who kindly corrected and improved the manuscript.

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ADENOCARCINOMA OF THE THYROID WITH HYPERTHYROIDISM AND FUNCTIONAL METASTASES

I. Studies with Thiouracil and Radio-Iodine[1,2,3]

LOUIS LEITER, M.D., PH.D., S. M. SEIDLIN, M.D., L. D. MARINELLI, M.A., AND E. J. BAUMANN, PH.D.

From the Medical and Laboratory Divisions, Montefiore Hospital, and the Physics Department, Memorial Hospital, New York, N. Y.

ADENOCARCINOMA of the thyroid with functioning distant metastases capable of producing clinical hyperthyroidism is exceedingly rare. In fact Frantz and her associates (7) state recently, "If such a case were ever found the study (that is, with radio-active iodine) might be most illuminating" (p. 687). Our good fortune in having the opportunity of observing two patients with this syndrome is the reason for this report. Subsequent papers will deal with the therapeutic and other effects of massive doses of radio-active iodine.

The story of thiouracil in experimental thyroid physiology and in the clinical treatment of Graves' disease has been well told (1, 6, 20, 31, 32, 39); but there has been no report on the effect of this drug on functioning carcinoma metastases. The present study furnishes clear evidence on thiouracil inhibition of the hyperthyroidism produced by carcinomatous thyroid tissue.

For the purposes of this study, the association of typical hyperthyroidism with a carcinomatous thyroid in the neck (or a documented history of its surgical excision) with metastases, showing evidence of function in their thyroxine content and their characteristic uptake and release of radio-active iodine (13, 14, 16), furnish the basis of the conclusion that the patient's hyperthyroidism is attributable to the neoplastic thyroid tissue. Furthermore, in one of our two patients, *B. B.*, there is no functioning thyroid tissue in the neck. Therefore, all thyroid hormone in this case must be derived from the active metastases [assuming, which may not be justified, (24, 26) that only thyroid tissue can form and release the thyroid hormone].

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[3] We are indebted to Dr. B. W. Carey of Lederle Laboratories, Inc., Pearl River, N. Y., for the supply of thiouracil.

CASE HISTORIES

L. H. (Montefiore Hospital *#36864*), aged 52, was admitted to the hospital on February 3, 1943. This patient, with a history of poorly treated lues, hypertension for many years and a right hemiplegia in 1935, was first admitted to another hospital in June, 1937, because of nervousness, sweating, choking sensations and an enlarging mass in the neck. A diagnosis of thyroid adenoma (left lobe) and hypertensive heart disease was made in December, 1939. The possibility of Graves' disease was excluded because the basal metabolic rate was only +18 per cent, and on iodinization fell only to +16 per cent. On exploration of the thyroid, the right lobe was found normal and was not excised. A large encapsulated mass, extending substernally on the left, was removed along with part of the main left lobe. Because the pathological report was "adenocarcinoma of the thyroid," the right lobe was again explored in February, 1940, and found normal. However, metastatic lesions developed in the left side of the neck and lungs in the following year. The former were excised but there were local recurrences. By November, 1942, the clinical picture of hyperthyroidism was well developed.[4] Roentgen therapy in dosage of 2000 r was given to the tumor in the neck with little effect. A weight loss of 18.2 kg. (40 lbs.) had occurred since December, 1939.

On admission in February, 1943, the patient showed a mass measuring 4 cm. slightly adherent, in the left supraclavicular region. There was also a residual mass in the left lobe of the thyroid. The patient had high grade myopia with left exophthalmos. There was no lymphadenopathy. Breath sounds over the left lobe were diminished. There was some cardiac enlargement to the left, and a systolic murmur was heard at the apex and aortic areas. The blood pressure was 190 mm. Hg., systolic; 80, diastolic; the pulse rate, 112. The skin was warm and dry; there was marked edema of the legs. The left shoulder was painful; there was no tenderness over other bones. The basal metabolic rates were +32 per cent and +39 per cent on February 25 and March 10, respectively. The patient's weight was 62 kilograms without edema. X-ray films showed round metastatic lesions in the left upper lung field, enlarged hilar nodes and possibly pleural metastases, numerous small lesions in the lower two thirds of the right lung field, and a metastatis in the outer third of the left clavicle with a pathological fracture. From March 14 to 17 the patient had an attack of pneumonia, for which sulfadiazine was given. On April 13, the basal metabolic rate was +36 per cent. The mass in the left supraclavicular region had increased in size to 6 by 5 cm.

Lugol's solution was begun on April 15, and from then until May 18 the patient received 2 cc. daily; from May 9 to June 8, 3 cc. daily. By April 30, the basal metabolic rate had risen to +71 per cent and remained between this figure and +80 per cent during the iodine therapy. Weight loss, nervousness and palpitation increased along with weakness. The objective data are shown in Figure 1.

Another roentgen treatment of 2000 r, directed to the mass in the neck, (May 8–June 4) had no apparent effect. By June, 1943, x-ray films showed in increase in the size and number of pulmonary metastases, and there was some osteoporosis of the skull and pelvis. In September an x-ray film of the left radius showed a lesion in the upper third with a pathological fracture; also, there was an extension of the metastases in the left chest wall and throughout the left lung, followed by a pleural effusion.

Biopsies of the tumor mass in the left neck were made on September 17, 1943, during

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[4] X-ray dosage is expressed throughout this paper as roentgens in air at the level of the skin portal. Tumor doses will be given in a later publication.

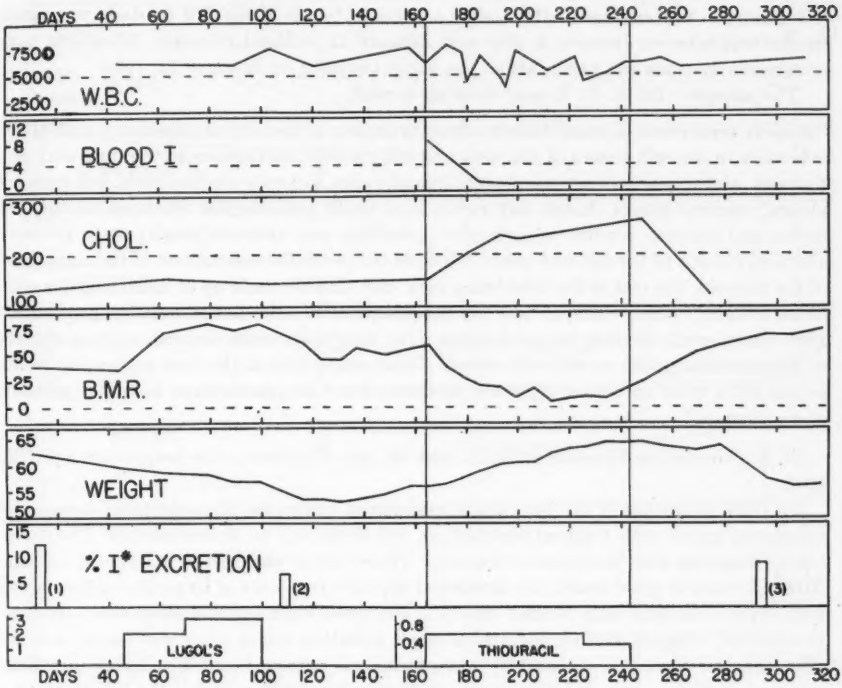

FIG. 1. Clinical data of patient *L. H.*

W.B.C.—white blood count (cells per cubic milimeter).

Blood I.—blood iodine (micrograms per 100 cc. of blood).

Chol.—total cholesterol (milligrams per 100 cc. of plasma).

Weight in kilograms.

%I*. Excretion—radio-iodine excreted (percentage of administered dose in first 96 hours).

(1) 0.6 millicuries of radio-iodine; 0.25 milligrams of inactive carrier iodine.

(2) 1.0 millicurie; 0.15 milligrams of inactive carrier iodine.

(3) 0.9 millicuries; 0.05 milligrams of inactive carrier iodine.

Lugol's (cc. per day).

Thiouracil (grams per day).

the thiouracil period, and again on December 23. The pathological report on the first biopsy (Dr. S. H. Rosen) was "small follicle type (with colloid) adenocarcinoma of the thyroid (malignant adenoma)." The second biopsy showed "small follicle adenocarcinoma with beginning papilloma formation" (Dr. David Marine). The iodine content of the two biopsy specimens was 0.29 mg. and 0.32 mg. per gram of dry tissue, respectively, with a thyroxine fraction of 17.9 and 21.5 per cent.

Following the discontinuance of thiouracil, the patient's hyperthyroidism rapidly recurred. Severe dyspnea set in, due to extension of pleural metastases and effusion. The

left chest was tapped repeatedly. Iodine administration in doses of 2 cc. daily was again carried out between January 3, 1944 and January 12, without response. After four days of extreme dyspnea and tachycardia, the patient expired on January 12, 1944.

The autopsy (Dr. S. H. Rosen) showed, in part,

"massive recurrence of small follicle adenocarcinoma at the site of lobectomy and direct extension to the soft tissues of the neck and left clavicle; metastases to the deep cervical, thoracic, abdominal and retroperitoneal lymph nodes, anterior mediastinum, left parietal pleura, visceral pleura, lungs, left radius and skull; pathological fractures of the left radius and clavicle; cardiac hypertrophy, arteriolo- and arteriosclerosis; early primary adenocarcinoma of the sigmoid colon; multiple old involuted adenomata of the right lobe of the thyroid, the rest of the lobe being very fibrotic and made up of small follicles with dense colloid; visceral pleurae and the parenchymae of all lobes of both lungs studded with many small, discrete tumor nodules. The lung metastases showed varying degrees of differentiation, with or without colloid. There was colloid in the bone metastasis in the radius but a more columnar type of epithelium, more anaplastic than the older portions of the tumor."

B. B. (Montefiore Hospital *#38213*), aged 50, was admitted to the hospital on April 20, 1942.

In 1923, at the age of 30, the patient underwent a subtotal thyroidectomy because of substernal goiter with tracheal obstruction, but there was no thyrotoxicosis. The histological diagnosis was "malignant adenoma." There was no postoperative hypothyroidism. After 15 years of good heatlh, he developed typical symptoms of hyperthyroidism along with severe, constant pain located chiefly in the lower back and radiating down the legs. In October, 1939, at another hospital, a small pulsating tumor mass was found over the 12th thoracic vertebra, apparently extradural and extraosseous. The basal metabolic rate was +40 per cent. A laminectomy in November, 1939 revealed the tumor to be a metastatic thyroid carcinoma. Histologically, there were small follicles, well differentiated, with colloid. The patient had a postoperative thyroid crisis. In the next two years, hyperthyroidism increased. In 1941, the basal metabolic rate was +45 per cent. X-ray films now showed round metastatic deposits in the lower lobes of both lungs. A gastrointestinal x-ray series was negative. There was also x-ray evidence of destruction of the right upper femur and of the second left rib. From December, 1941, to February, 1942, a total dose of 6800 r of roentgen radiation was directed to the dorsal and lumbar spine and the right upper femur. X-ray films of the pelvic bones eventually revealed osteolytic lesions and an extraosseous lesion within the pelvis. A lesion in the skull was also found.

On admission in April, 1942, the patient complained of pain in the lower spine, in addition to symptoms of hyperthyroidism. Examination showed a rather small, emaciated, poorly developed individual, aged 48, whose weight was 28 kg., height, 146 cm.; he was hyperkinetic; the blood pressure was 128 mm. Hg., systolic, 50, diastolic; pulse rate, 100–110 per min. There were no eye signs. No thyroid tissue was palpable in the neck. The heart was normal and the lungs were clear. There was a laminectomy scar and tenderness over the lumbar spine The skin was warm, and there was a coarse tremor of the hands. The basal metabolic rate was +23 per cent on April 27 and +36 per cent on May 7, 1942.

In view of the evident hyperthyroidism, Lugol's solution was begun on June 13, and continued in dosage varying from 2 to 6 cc. daily until November 10 (Figure 2). The diet was increased in calories and supplemented with vitamins. The weight increased to 39 kg. by August. The basal metabolic rate was +30 per cent. By September 9, the rate had fallen to +18 per cent and remained at about +25 per cent for the next three months.

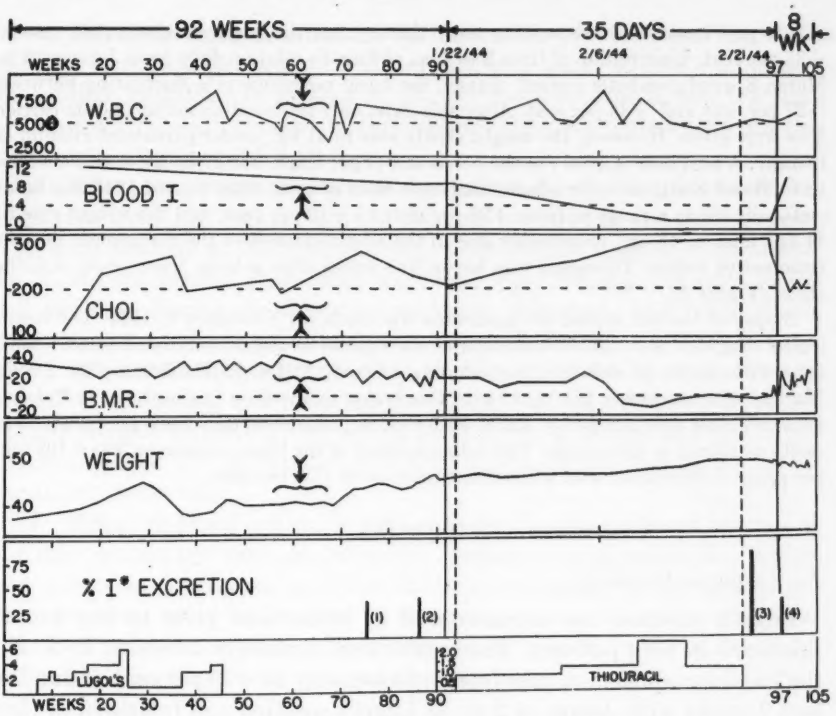

FIG. 2. Clinical data of patient *B. B.*

W.B.C.—white blood count (cells per cubic millimeter).
Blood I.—blood iodine (micrograms per 100 cc. of blood).
Chol.—total cholesterol (milligrams per 100 cc. of plasma).
Weight in kilograms.
%I*. Excretion—radio-iodine excreted (percentage of administered dose in first 96 hours).

(1) 2.0 millicuries of radio-iodine; trace of inactive carrier iodine.
(2) 5.0 millicuries; 0.3 milligrams of inactive carrier iodine.
(3) 0.5 millicuries; 0.03 milligrams of inactive carrier iodine.
(4) 1.5 millicuries; 0.1 milligrams of inactive carrier iodine.

Lugol's (cc. per day).
Thiouracil (grams per day).
Heavy arrow indicates administration of therapeutic dose of radio-iodine.

The plasma cholesterol rose from 114 mg. to 230 mg. per cent. The weight rose further to 45 kg. However, in spite of some remission of hyperthyroidism, the bony lesions grew and new ones appeared in the left ischium and right femur. Little relief resulted from another 4750 r of x-radiation given over the pelvis and upper femur from December 9, 1942, to January 6, 1943. Between November 11, 1942, and January 8, 1943, only 1 cc. of Lugol's solution was given daily. The observed fall in weight to 38 kg., was difficult

to interpret because of overlapping x-ray therapy but the basal metabolic rate rose to +35 per cent. Resumption of from 3 to 4 cc. of Lugol's solution daily from January 9 to March 8, produced little clinical change, the basal metabolic rate fluctuating between +27 per cent and +35 per cent. After this date, only radio-active iodine and its carrier dose were given. However, the weight slowly rose to 41 kg., under parenteral vitamin B therapy. A final dose of 3000 r to the pelvis and upper thigh, was given between February 18 to March 8 with no other effects than some relief of pain. After August 1943, the basal metabolic fell to a range of from +25 per cent to +10 per cent, and the weight rose to 44 kg., later to 46 kg., presumably due to the administration of the therapeutic dose of radio-active iodine. Thiouracil was begun five weeks after a large tracer dose, 5 millicuries (Figure 2).

Biopsy of the left second rib metastasis was made on November 6, 1943. The histological diagnosis was "adenocarcinoma of the thyroid of the differentiated small follicle type with traces of colloid.—"metastasing adenoma" (Dr. S. H. Rosen—Dr. David Marine). Comparison of this tissue with the section removed on laminectomy in October 1939, revealed close similarity. Radio-active iodine, administered a week previously, was easily measured in the sample. The iodine content of the biopsy specimen was 0.192 mg. per gram of dry tissue, with a thyroxine fraction of 17.4 per cent.

RESULTS

A. Response to iodine

Lugol's solution was administered at some time prior to the use of thiouracil in both patients. The results were strikingly different. In *L. H.*, the basal metabolic rate rose from +34 per cent to +71 per cent within the first 2 weeks with dosage of 2 cc. of Lugol's solution and remained at that level in spite of increase of the dosage to 3 cc. daily, given for three weeks (Figure 1). The patient's general condition and weight curve also reflected the increase in hyperthyroidism. Shortly after discontinuance of iodine, the basal metabolic rate fell to about +55 per cent where it remained for the 7 weeks immediately preceding the administration of thiouracil (Figure 1).

In contrast, were the results in patient *B.B.*, (Figure 2) whose basal metabolic rate fell from about +32 per cent to about +20 per cent during the three months in which large doses of Lugol's solution were given. A gain in weight of 6 kg. occurred. The sudden reduction of the dosage from 6 cc. to 1 cc. daily was followed by a reversal of the metabolic rate and weight curve, uninfluenced by subsequent increase in iodine dosage over a period of two months.

B. Response to thiouracil

In both patients, the effects of ordinary doses (0.6 to 1.2 gm.) of thiouracil were dramatic. Apart from obvious subjective improvement, several objective criteria were available, such as, the basal metabolic rate, the plasma cholesterol, the body weight, and the blood iodine level (Figures 1 and 2).

(1). **Effect on basal metabolism.** In Patient *L. H.*, the basal metabolic rate fell from +58 per cent to +20 per cent in 3 weeks and to +9 per cent in another fortnight. In patient *B. B.*, the basal metabolic rate fell from +18 per cent to −12 per cent in three weeks, then fluctuated between this point and ±0 per cent for 10 days.

(2). **Effect on plasma cholesterol.** In Patient *L. H.*, the plasma cholesterol rose from 150 mg. per cent to 235 mg. per cent in the first 4 weeks. The level was still at 262 mg., five days after discontinuance of thiouracil. In *B. B.*, the plasma cholesterol increased from 206 mg. per cent to 252 mg. per cent during the first two weeks on thiouracil, and reached 295 mg. per cent at the time of the lowest metabolic rate.

(3). **Effect on body weight.** Patient *L. H.*, gained 6.4 kg. in the first 10 weeks on thiouracil, and later, another 2 kg., totaling 15 per cent of her control weight. Patient *B. B.*, with a control rise in weight of 3 kg. in two months, gained 2.4 kg. rapidly only after the maximum depression of the basal metabolic rate.

(4). **Effect on blood iodine.** In both patients the control blood iodine was 8.4 micrograms per cent, or twice the average normal value (2). Within two weeks, in *L. H.*, the level had fallen to less than 0.8 micrograms per cent, less than could be detected by the method used (2), and remained at that level at least five weeks after the thiouracil period. In *B. B.*, the blood iodine fell to less than 0.8 micrograms per cent.

C. Response to withdrawal of thiouracil

In both patients there was reversal of the objective effects of thiouracil when the drug was discontinued (Figures 1 and 2).

(1). **The basal metabolic rate** in *L. H.*, began to rise when the dosage of thiouracil was reduced to 0.4 gm. daily, increased rapidly and continuously after withdrawal of the drug, reaching the control figure of +55 per cent within four weeks. In *B. B.*, the basal metabolic rate showed little change in the first three weeks, then rose irregularly to the control level of +15 per cent within six weeks after thiouracil was discontinued.

(2). The **plasma cholesterol** in *L. H.* fell from 262 mg. to 123 mg. per cent within four weeks after thiouracil was stopped. In *B. B.*, the level droped from 300 mg. per cent to 235 mg. per cent in three weeks and to 200 mg. per cent in two more weeks.

(3). The **weight** was stationary for about a month in both cases. Subsequently, it fell rapidly to the control level in *L. H.*, but in *B. B.*, only half of the modest gain was lost slowly.

(4). The **blood iodine** was estimated only infrequently after the withdrawal of thiouracil. It was still below the level of detectability of 0.8 micrograms per cent a week later in *B. B.*, and a month later in *L. H.* In

B. B., however, the value had reached 9.4 micrograms per cent within five weeks.

D. The urinary excretion of radio-active iodine

The data are shown in Figures 1 and 2. They may be considered separately as the control pre- and post-thiouracil periods, and the period of thiouracil administration.

(1). During the **pre- and post-thiouracil periods** adequate data are available on both patients. In *B. B.*, there was an average urinary output of about 30 to 40 per cent of the tracer doses in 72 to 96 hours. This control level was re-established within 18 days after thiouracil was discontinued. In *L. H.*, the excretion was only about 12 per cent in 96 hours, in the pre-thiouracil period. This control level was found seven weeks after withdrawal of thiouracil.

(2). **On the first day of administration of thiouracil,** in patient *B. B.*, the excretion of radio-active iodine from a dose given five weeks previously was still about 0.67 per cent and remained so for two more days. Afterward, the output increased to 1.2, 1.4, 0.9 and 0.9 per cent per day. A small tracer dose (500 microcuries) was given on the day of withdrawal of thiouracil. The excretion was 79 per cent of this dose in the first 24 hours, and 86 per cent in the first 96 hours, or more than twice the control rates. No data are available for the thiouracil period of patient *L. H.*

E. Effect of thiouracil on the leucocyte count

The blood was carefully observed during the period of administration (Figures 1 and 2). In *L. H.*, the control leukocyte count was ordinarily from 6000 to 7500 per cu. mm. It fell to 4300 per cu. mm. on the 18th day of therapy but two days later it was 6850 per cu. mm. Other drops occurred at intervals of about two weeks with spontaneous return to normal. The percentage of polymorphonuclear leukocytes was constant throughout. In *B. B.*, the control white counts were usually from 5000 to 6300 per cu. mm. During the thiouracil period, the count ranged from 5050 to 8400 per cu. mm., with a normal differential. It fell to 4150 per cu. mm. when the patient was receiving, by error, 2.4 grams of thiouracil daily. Resumption of the dose of 1.2 gm., was followed by a prompt return to a normal count. In this case, the total period of thiouracil treatment was only 30 days in contrast to the 78 days in case *L. H.* It should be noted that *L. H.* was receiving crude liver extract intramuscularly, in addition to triple B vitamins and vitamin C orally. *B. B.*, had triple B vitamin parenterally, but no liver.

DISCUSSION

The frequency of carcinoma of the thyroid with metastases, but without hyperthyroidism (4, 7, 37), makes the isolated cases with hyperthyroidism all the more intriguing. The latter group should not include instances of Graves' disease due to an ectopic thyroid tumor of the ovary (10, 17, 30). More pertinent may be the case with a functioning metastasis in bone described recently by Dickson and associates (5).

Hyperthyroidism associated with tumors of the thyroid has often been attributed to activity of the original thyroid tissue (4, 28). Unfortunately, many of the reports merely involve hyperplastic glands of Graves' disease found, on histological examination, to contain adenomas or small carcinomas. Goetsch has recently criticized the concept of primary carcinoma in the hyperplastic thyroid (9). The lack of demonstrable correlation between carcinoma of the thyroid and clinical hyperthyroidism has been emphasized in a review of extensive surgical material by Pemberton (28) and by Ward (38). The latter author found only one case of carcinoma in a "diffuse toxic goiter" among 168 of cancer of the thyroid. Conversely, Crile (3) reported only one case of hyperthyroidism among 249 with thyroid neoplasms. The difficulties in the analysis of this problem are well illustrated by a recent paper of Friedell (8). After a critical discussion of the endocrine activity of thyroid tumors, Lerman (18) concludes (p. 271):

> The association of hyperthyroidism and thyroid cancer is rare. There is no evidence that thyroid malignancy can produce an excess of thyroid hormone or that hyperplastic tissue can become malignant. It is more likely that malignancy arises in a pre-existing adenoma imbedded in the hyperplastic gland.

The two cases now reported are unique in showing the association of adenocarcinoma of the thyroid, functioning metastases widely distributed in the osseous and pulmonary systems, and clinical hyperthyroidism. Patient *B. B.*, having undergone a thyroidectomy for a "malignant adenoma" in 1923, apparently has no functional thyroid tissue in the neck, as shown by the lack of Geiger-Müller counter response in that vicinity. Therefore, the hyperthyroidism must be attributed entirely to excessive hormone release from distant metastases. Furthermore, every demonstrable metastasis in this case is apparently capable of radioiodine retention and hormone production to some extent. (See page 250 for the thyroxine content of tumor biopsy.) Patient *L. H.*, did not present so clear a picture. She still had a tumor-free right lobe of the thyroid, capable of storing radioactive iodine but histologically atrophic with old, multiple adenomata. At autopsy, it contained 2.14 mg. I_2/gm. (dry), with a thyroxin fraction of 24.2 per cent. On the other hand, the recurrent tumor in the left neck and

several of the distant metastases did pick up and retain radioiodine and, therefore, could have been a source of excessive thyroid hormone. Numerous metastatic nodules, however, were anaplastic morphologically and nonfunctional physiologically, judging from their lack of radioactivity.

The usual situation is probably represented by the series of four cases reported by Hamilton and associates (11, 13), in which the cancerous portion of the thyroid failed to take up or store radioactive iodine in contrast to the normal tissue. The patient studied by Frantz and her associates (7, 16), the first to be reported with storage of radioactive iodine in a metastasis, had numerous non-functioning metastases and never showed clinical hyperthyroidism.

The effect of thiouracil on the hyperthyroidism associated with functioning metastases resembled in every respect the response of ordinary thyrotoxicosis or Graves' disease (1b, 1c, 23, 25, 27, 31, 34, 39). Furthermore, the ability to retain tracer doses of radioactive iodine was markedly impaired in the one patient on whom this measurement was made. There is every reason to assume that the other patient would have behaved similarly. None of the various serious reactions described after the use of thiouracil (1b, 1c; 15, 23, 35, 39) occurred in our two patients although one of them, *B. B.*, was given 2.4 gm. a day for four days through an error. Transitory falls in leukocyte count to 4500 and 3800 per cu. mm. occurred in both patients, with spontaneous return to the control levels of 6000 to 8000 per cu. mm.

That thiouracil was responsible for the observed changes was shown by the effect of withdrawal of the drug on the basal metabolic rate, the body weight, the blood iodine, the plasma cholesterol, the general clinical status in both patients, as well as the ability to retain radioactive iodine, determined only in patient *B. B.* The return to the pre-thiouracil status occurred within a few weeks. Here, again, there is a striking similarity to the situation in ordinary Graves' disease when treated with thiouracil over a short period (1b, c; 34).

An interesting point in this connection is the effective dose of thiouracil (3c). From the number and volume of metastases known to be functioning in *B. B.*, one might have anticipated that it would require more than the 0.6 to 1.2 gm. of thiouracil daily to control the hyperthyroidism. Evidently, even this amount is adequate for several hundred grams of mildly active thyroid tumor tissue. However, in *L. H.*, with severe hyperthyroidism, reduction of dosage to 0.4 gm. daily was followed in two weeks by a definite rise in metabolic rate.

Thiouracil was completely effective in both patients in contrast to iodine which aggravated the hyperthyroidism of L. H., (See Figure 1) while exerting only a slight restraining influence in *B. B.* The superiority of thiouracil

over iodine in inducing a complete remission in ordinary Graves' disease is now well established (1, 25, 27, 31, 39). It is presumably inherent in the basic nature of thiouracil action, which is to suppress thyroid hormone formation by blocking some enzymatic process necessary for the conversion of inorganic iodine to thyroxine (1e, 1d, 6, 32).

The relationship between the basal metabolic rate and the blood iodine after the discontinuance of thiouracil was not a simple one. In *L. H.*, severe hyperthyroidism was re-established in spite of a level of blood iodine less than 0.9 micrograms per cent persisting for five weeks. Yet, a biopsy of her tumor tissue after another three weeks showed 0.32 mg. of iodine per gram of dry tissue and a thyroxine fraction of 21.5 per cent. Normal or even low blood iodines have been reported in thyrotoxicosis, especially in long standing cases (29, 36). Methods of determination are subject to criticism (33). Our patients were deliberately kept on diets relatively low in iodine. It is conceivable that after cessation of thiouracil inhibition there may be a relative "vacuum" for iodine in the thyroid and for thyroid hormone in the peripheral tissues, resulting in a low blood iodine until "saturation" occurs. Under these conditions, a direct correlation between the protein-bound iodine of the plasma and the basal heat production (19) would be surprising.

The uptake of radio-active iodine and its rate of excretion in the urine were essential criteria of the functional state of the thyroid metastases. In patient *B. B.*, with a "subtotal" thyroidectomy, it was impossible to demonstrate any uptake of radio-active iodine in the region of the thyroid. In patient *L. H.*, a left lobectomy and several partial excisions of recurrent tumor had been performed. Data on the uptake of radio-active iodine by the right lobe of the thyroid were difficult to interpret because of the proximity of the large functional mass in the left side of the neck. However at autopsy the right lobe of the thyroid was definitely atrophic and did contain radio-iodine, and 2.14 mg./gm. total iodine with a thyroxine fraction of 24.2 per cent, although the patient died in the course of a severe hyperthyroidism three months after the thiouracil was discontinued. One must attribute her hyperthyroidism to the large functioning metastases.

The control values for the urinary excretion of radio-active iodine in our patients, from 30 to 40 per cent of the administered dose in *B. B.*, and 12 per cent in *L. H.*, in from 72–96 hours, correspond roughly with those obtained in uniodinized classic Graves' disease with similar doses (14b, 14d). Normal subjects have yielded urinary outputs of 60 per cent (14 b) and 80 per cent (12, 31). Frantz and her associates (7) found 52 per cent excretion in 48 hours in their case of thyroid tumor metastases, but without hyperthyroidism. The response to thiouracil treatment in our patient, *B. B.* was the expected one, more than double the output of the control period. This may be compared with the corresponding data of 55–93 per cent on

ordinary hyperthyroidism under thiouracil management (31). The increased excretion of the tracer dose is the reflection of the impairment of thyroxine formation under the influence of thiouracil. The low control excretion rate of 12 per cent in patient *L. H.*, is probably a direct index of the severity of her hyperthyroidism.

The permanence of thiouracil's therapeutic effect was not determined in our patients. One of them, *L. H.*, died chiefly as the result of extensive pulmonary metastases, most of which were anaplastic and non-functional. The other, *B. B.*, has since been given massive therapeutic doses of radioactive iodine and is still under investigation.

No evidence is available as to the morphological effects of thiouracil on the thyroid metastases comparable to the published data on non-neoplastic thyroids in man, (25, 31, 34, 39b). It would probably be difficult to interpret observed changes in tumor biopsies before and after thiouracil. In the case of *L. H.*, no essential histological difference was noted in biopsies made during the five weeks after thiouracil treatment. In the case of *B. B.*, x-ray films failed to reveal any difference in the number or apparent size of lesions. The problems of the effect of prolonged thiouracil administration on the rate of growth of thyroid metastases and on possible conversion from relatively benign to more anaplastic growth or *vice versa*, must be left for future study. It is significant that the hyperplasia during the use of thiouracil is dependent, like other hyperplasias of the thyroid (21a) or of thyroid transplants (22), on the action of thyrotropic hormone (1a, 1e, 20, 24).

The factors determining whether or not carcinomatous thyroid tissue functions or "hyperfunctions" are probably related to the various mechanisms prevailing in the normal individual and in cases of Graves' disease, on the nature of which there is as yet considerable difference of opinion (21b, 24). In this regard, the use of thiouracil and radioactive iodine, especially in correlation with histological data, may furnish valuable evidence.

SUMMARY

1. Two cases of adenocarcinoma of the thyroid with functioning metastases and hyperthyroidism have been studied for the first time with radioactive iodine as an indicator of the physiological activity of the metastases. In one patient, the absence of functional thyroid tissue in the neck, shown by the lack of uptake of radio-active iodine, proved that the metastases were responsible for the hyperthyroidism. Thyroxine iodine was demonstrated in metastases in both patients.

2. Thiouracil produced a complete remission in both patients within a few weeks, as judged by the effect on the general condition, the basal metabolic rate, the plasma cholesterol, the body weight and the blood

iodine. This was in striking contrast to the aggravating effect of previous iodinization in one patient and the slight decrease in hyperthyroidism of the other.

3. Upon withdrawal of thiouracil, there was prompt recurrence of hyperthyroidism in both patients.

4. The plasma cholesterol curve was a sensitive indicator of the influence of thiouracil.

5. The urinary excretion of radio-active iodine was doubled during the period of maximum effect of thiouracil.

6. From these results it may be concluded that thiouracil can suppress hormone production in metastases of adenocarcinoma of the thyroid, just as readily as it inhibits this process in the hyperplastic thyroid of ordinary Graves' disease.

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MIKULICZ'S SYNDROME WITH FEVER AS AN UNUSUAL COMPLICATION OF THIOURACIL THERAPY

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"IODINE MUMPS" has long been known to the medical profession as a rare, toxic reaction to iodine (1). Indeed iodine has been reported as an etiologic factor in some cases of Mikulicz's syndrome (3). An analogous phenomenon has been reported to occur in patients receiving thiouracil in the treatment of thyrotoxicosis (4). The present report deals with the detailed description of a patient who developed fever and a generalized swelling of the salivary and lacrimal glands while taking thiouracil. Following recovery, a sialogram showed evidence of structural damage to at least one of the salivary glands.

CASE REPORT

A. L., Lab. #25523, a white female aged 50, was admitted to the medical service of the Massachusetts General Hospital on February 23, 1945. She complained of a goiter, weight loss and nervousness.

In 1942 she first noticed in her neck a lump which produced no symptoms. At about the same time she was treated in another hospital with x-ray and radium for epidermoid cancer of the cervix. In July, 1944, she consulted a physician and complained of fairly classic symptoms of thyrotoxicosis. Lugol's solution was prescribed and symptomatic improvement followed. However, the improvement was transient and by February of 1945 she had lost 9.1 kg. (20 lbs.) in weight. The iodine was stopped and she was referred to this clinic.

Positive physical findings at the time of entry were evidence of weight loss, a diffusely nodular goiter which was from three to four times the size of a normal thyroid, and a pulse rate of 120 per minute. No bruit was heard over the thyroid. Laboratory examinations revealed a normal erythrocyte count and hemoglobin; the leucocyte count was 5,400 per cu. mm., and the differential count was 64% neutrophils, 27% lymphocytes, 5% monocytes, 2% eosinophils and 1% basophils. The urine was negative. The level of values of the basal metabolic rate before treatment was +27%.

It was thought that the patient could best be prepared for thyroidectomy with thiouracil, although it was recognized that the previous treatment with iodine might retard the response. Accordingly thiouracil in a dose of 0.2 grams every six hours was started on February 27, 1945. The patient also received a preparation of crude liver (Klein's Liver Fraction L).

Her course was uneventful until the 16th day of treatment with thiouracil, when a sore throat and general malaise developed. Definite swelling and induration of both submaxillary glands with a local erythema appeared on the 18th day of treatment. By this time the temperature had risen to 39.7,C. (103.6,F.). The leucocyte count was 11,600 per cu.

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mm. with a differential count of 80% polymorphonuclear cells. It was thought that the physical signs together with the leukocytosis and fever were due to a streptococcal infection, although a throat culture taken on the sixteenth day had not been reported. Thiouracil was continued and treatment with sulfadiazine was begun. The submaxillary swellings remained and the tissue of the floor of the mouth became reddened and indurated. On the twenty-third day of treatment with thiouracil preauricular swelling and induration appeared. The orifices of Stenson's ducts were not inflammed. The throat culture was reported negative for beta hemolytic streptococcus. At this time it was postulated that the patient was suffering from a drug reaction. Thiouracil and sulfadiazine were discontinued and penicillin was given intramuscularly in a daily dose of

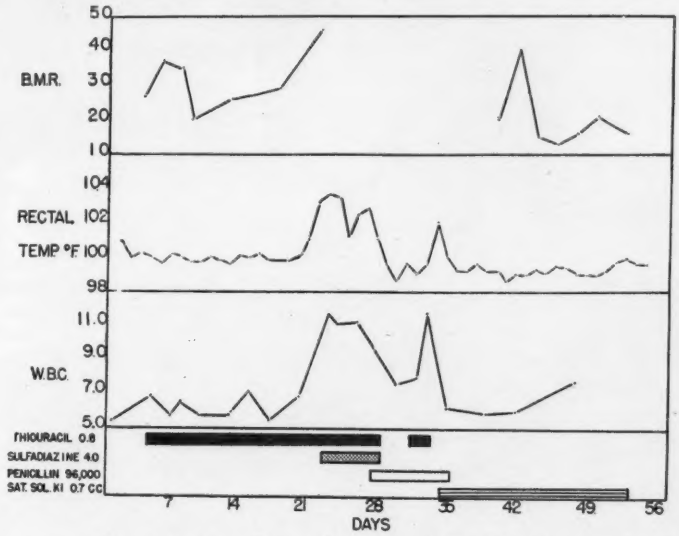

FIG. 1. The effect of thiouracil on temperature and white blood count. It will be noted that withdrawal of thiouracil resulted in a fall in the temperature and in the white blood count. When the drug was exhibited the second time, fever and leukocytosis recurred but subsided promptly.

96,000 units. There was a rapid subsidence of the fever, salivary gland involvement, and the leukocytosis. Within four days a small amount of submaxillary swelling was the only residual of the toxic reaction.

After the patient had remained afebrile for 48 hours test-doses of thiouracil of 0.2 gm. were administered twice, six hours apart, to confirm the diagnosis of drug reaction. Within three hours after administering the second test-dose of thiouracil the patient had experienced a sore throat and malaise and the temperature had risen to 37.8, C. (102, F.) (see Figure 1). By the following morning there was marked swelling of the parotid, submaxillary and lacrimal glands. A purulent exudate containing many polymorphonuclear leukocytes and an occasional gram positive coccus could be expressed from both of Stenson's ducts. A culture of the exudate showed a few alpha hemolytic streptococci and a rare colony of staphylococcus aureus. The leucocyte count rose to 11,700 per cu. mm. with a proportion of 78% polymorphonuclear leukocytes.

The temperature returned to normal, and the symptoms of sore throat and malaise were gone by the following day. Swelling of the salivary glands disappeared gradually. However, dryness of the mouth, due to decreased salivary secretion, persisted as a troublesome symptom for more than two weeks after stopping the thiouracil.

Preparation for thyroidectomy was continued with the administration of a saturated solution of potassium iodide. Her basal metabolic rate fell to plus 16% in 18 days. The

FIG. 2. Sialogram of the right parotid made seventy days after the onset of the first reaction.

thyroid was removed in two stages. The first hemi-thyroidectomy was performed on April 18th and the second on April 26th, 1945. Convalescence was uneventful.

The patient returned to the Out Patient clinic on May 25, 1945. She felt very well and was free of symptoms of thyrotoxicosis. The salivary glands were not enlarged nor tender. Though she did not complain of dryness of the mouth, the oral mucous membranes appeared dry. No saliva could be expressed from Stenson's duct on that side. After the right duct had been cannulated a sialogram was made of the right parotid gland by Dr. David Weisberger. This procedure revealed narrow salivary ducts and increased filling of the alveoli (Figure 2). Dr. Weisberger interpreted these findings as indicating atrophy of the right parotid gland.

DISCUSSION

That the fever, leukocytosis and swelling of the salivary glands in this patient represent a toxic reaction to thiouracil seems clearly established by reproduction of the syndrome on readministration of thiouracil.

The unusual reaction observed in this patient is one of fifteen toxic reactions observed in one hundred and four patients treated with thiouracil at the Massachusetts General Hospital. It is one of five cases of fever which were attributed to thiouracil.

McArthur, Rawson and Means (2) from this clinic have reported the other cases of proven drug fever. They have suggested that the febrile reactions may be due to sensitization to a thiouracil-protein complex. Such an explanation might account for the fever observed in the present case. However, the salivary gland changes are probably ascribable to a different mechanism.

There is no available information as to the content of thiouracil in the saliva. However, since Williams, Kay and Jandorf (5) reported it to be contained in body fluids tested by them, it seems quite likely that thiouracil is also present in the salivary secretion. One could then postulate that in the process of being excreted in the saliva thiouracil may cause damage to the parenchyma of the salivary gland. Whether or not the drug damages these tissues probably depends on the state of the gland prior to any administration of the drug.

SUMMARY

An unusual toxic reaction to thiouracil consisting of swelling of the salivary and lacrimal glands, fever and leukocytosis is reported.

A sialogram of one parotid made seventy days after the onset of the reaction showed evidence of atrophy of that gland.

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IDIOPATHIC HYPOPARATHYROIDISM WITH MENTAL DETERIORATION: EFFECT OF TREATMENT ON INTELLECTUAL FUNCTION

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IDIOPATHIC hypoparathyroidism, though rare, is a disease with many interesting aspects. The characteristics of the disturbances in calcium and phosphorus metabolism were described by Albright and Ellsworth (1) in 1929. Additional important clinical and pathological observations were presented by Albright and associates (4) in a rather full review of the subject in 1939. The essential considerations in the treatment of hypoparathyroidism were clarified by McLean (10) in 1941.

Of particular interest are the severe cerebral changes so frequently associated with this condition. Psychotic reactions have been observed in idiopathic parathyroid insufficiency (8) as well as in the postoperative type (9). The concomitant occurrence of intracerebral calcification, convulsions, and mental deterioration has been emphasized by Eaton *et al* (5, 6). Barr, MacBryde, and Sanders (2), and others (14), have shown that increased intracranial pressure and papilledema may accompany parathyroid tetany. The relationship of these varied disturbances to the underlying hypoparathyroidism is unknown.

Although intellectual impairment is a frequent finding in idiopathic hypoparathyroidism, very little has been written concerning the origin of this defect and its response to treatment (6). In the case to be presented particular attention was directed to this aspect of the problem.

CASE REPORT

V. R., a 19 year old, white, single female, was admitted to the University Hospital on Jan. 6. 1945. The complaint was "drawing of the muscles and unconscious spells." The first symptoms appeared at the age of eight years. While running the patient suddenly fell flat, was unable to get up for several minutes, but then resumed activity. A few months later, while on the way to the dentist, her legs became stiff and awkward, and she had to be held up. Her expression was staring and she could not speak for a short time. At the age of ten the patient had a fainting spell which lasted 30 minutes but there were no unusual motor phenomena. During the following year the patient noted frequent attacks of stiffness of her hands, which would assume the typical accoucheur's position. Around the age of 11 she began having generalized convulsions. These recurred every

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three or four months for the next several years and necessitated her removal from school at the age of 13, when beginning the seventh grade. At the age of 14 she was given some liquid medicine containing calcium. This preparation abolished all symptoms for approximately one year but after that it was no longer effective and her illness progressed in severity. About nine months prior to admission all the symptoms became much worse. The convulsions occurred several times weekly and the tetanic manifestations were evident most of the day. For two months prior to admission the patient had been confined to bed since any slight exertion would precipitate a convulsive seizure.

During the course of her illness the family had noted a progressive personality change. The patient developed considerable nervousness and irritability. Her temper became extreme in contrast to her usual complacency. She became increasingly stubborn and demanding as well as childish and simple.

Delivery was at term and was spontaneous. Development was entirely normal in childhood. The patient had whooping cough shortly after the onset of tetanic symptoms and the parents felt this had aggravated her difficulty. The menses began at age 12 and were normal in all respects until the age of 16, when they became decreased in frequency and in the amount of flow. The patient was the fifth of seven children. Five of the siblings were living and well; one died at the age of five months of "colitis." The father and mother were living and well. The family history was entirely negative for any significant diseases, including convulsive disorders.

Physical Examination

The patient was a well developed, moderately obese, white female. The skin, hair, and nails were entirely normal. There were some small, discrete opacities in both lenses, as seen with the ophthalmoscope. The optic fundi showed increased tortuosity of the veins. The optic discs were slightly elevated. The teeth showed good enamel formation but were spaced and peg-shaped. Numerous teeth were missing. The thyroid was not palpable. The heart was normal to percussion and auscultation. The blood pressure was 110 mm. Hg., systolic, and 80 mm. Hg., diastolic. The deep reflexes were all absent. The abdominal reflexes were present but sluggish. There were no pathological reflexes. The muscle tonus was not increased in the intervals between attacks of carpal spasm. Chvostek's sign was very easily elicited. Trousseau's sign was present after the tourniquet was applied for 90 seconds. A diligent search revealed no evidence of Monilia infection.

Laboratory Examinations

The very complete laboratory studies gave normal values, except for the following: serum Ca, 4.5 mg. per 100 cc.; serum inorganic P, 9.6 mg. per 100 cc.; serum alk. phosphatase, 5.6 Bodansky units. The spinal fluid pressure was 240 mm. of H_2O.

Roentgenograms of the skull showed bilaterally symmetrical areas of punctate calcification (Figure 1) consisting of small, discrete, irregular deposits. A pneumoencephalogram was interpreted as showing a normal ventricular system and normal cortical pathways. The fine stippled calcium deposits were seen to be in the brain substance in the region of the basal ganglia. The inner and outer tables of the skull were increased in thickness with narrowing of the diploe. In other respects the bone structure was interpreted as within normal limits. Films of the right ankle and thorax did not reveal any increased density of the bone. Films of the teeth disclosed that twelve molars and bicuspids were unerupted and growing at bizarre angles.

Slit-lamp examination: each lens contained innumerable dust-like and punctate opaci-

ties intermingled with larger flakes. They were of whitish color and were distributed throughout the lens substance.

With galvanic stimulation of the right median nerve the cathodal opening contraction took place with a current of 1.2 ma.

Pseudo-hypoparathyroidism was excluded by the Ellsworth-Howard test (7). Following the injection of parathyroid extract (*Lilly*) there was a marked increase in the excretion of phosphorus (Table 1).

TABLE 1. ELLSWORTH-HOWARD TEST

Time	Urine		Serum	
	Volume	P	Ca	P
hour	cc.	mg.	percent Mg.	percent Mg.
7– 8	140	14		
8– 9	540	34		
9–10	350	33		
10:30 2 cc. Parathyroid Extract, I.V. (200 units)			4.6	9.6
10–11	180	26		
11–12	380	100		
12– 1	190	42		
1– 2	210	49	4.2	7.9

ELECTROENCEPHALOGRAMS

Using a three lead, Grass electroencephalograph, a tracing was taken on Jan. 8, 1945. The serum calcium level at this time was approximately 5 mg. per 100 cc. With leads attached to the right frontal, parietal, and occipital areas and the patient at rest, the tracing (Figure 2A) revealed a very gross dysrhythmia. There was a great deal of fast activity with frequent spikes and bursts of Beta rhythm. The amplitude ranged from 15 to 60 microvolts. Hyperventilation (Figure 2B) exaggerated this pattern and introduced much slow activity (3 to 5 per second waves). There was no difference in the tracing as recorded from the right and left hemispheres.

Another recording was made on Feb. 10, 1945. At this time there was complete freedom from tetanic symptoms. The serum calcium was 8.1 mg. per 100 cc. on Feb. 9, 1945. This tracing showed a marked improvement (Figure 3A). The voltage was reduced to normal. The fast spiking activity persisted to a slight degree in the frontal areas. Alpha rhythm appeared in the occipital areas. Hyperventilation (Figure 3B) brought out a moderate amount of slow activity. In general the tracing was only slightly irregular.

PSYCHIATRIC EXAMINATION

The patient was a well built, moderately obese, white female who appeared younger than her stated age. She was reasonably content with her invalid existence but spent a great deal of time and effort on her personal appearance. She was oriented and coopera-

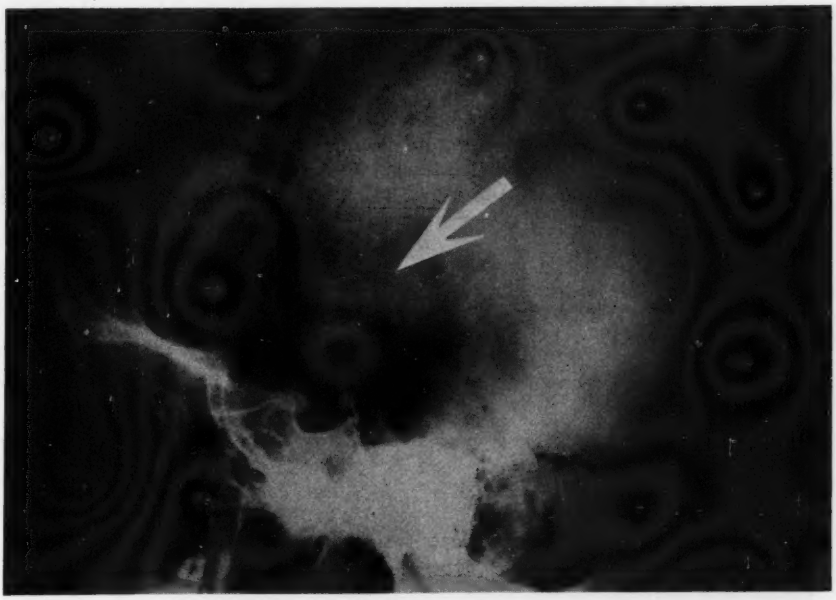

FIG. 1. Roentgenogram of skull showing calcification in region of basal ganglia.

tive, responded readily to questions, but rarely volunteered any information. The range of interests was very narrow and centered exclusively upon herself. Her emotional reactions were rather immature and childish. There was no significant alteration of mood nor abnormal mental content. The attention, comprehension, and memory were somewhat impaired. The fund of general knowledge was strikingly limited.

On Jan. 29, 1945, the patient was given several standardized psychological tests. This was at the onset of treatment, before there had been any clinical or laboratory improvement. The I.Q., as determined by the 1937 Revision of the Stanford-Binet Scale, Form

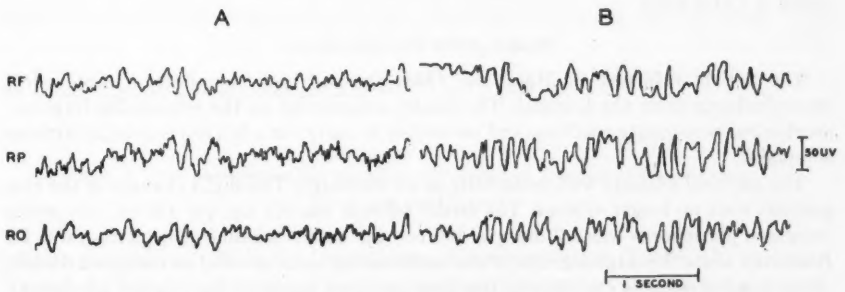

FIG. 2. Electroencephalogram before treatment was begun (A—at rest; B—hyperventilation).

L, was 70, with a mental age of ten years, six months. The full I.Q. on the Wechsler-Bellevue Intelligence Scale was 79 (Verbal I.Q.—80, Performance I.Q.—82). This nine point difference between the Stanford-Binet and Wechsler-Bellevue Scales was not considered to have great significance. It was probably due to the fact that lack of education is a greater handicap on the Stanford-Binet than on the Wechsler-Bellevue Scale. The latter test appeared to be the more accurate measure of her level of function. Evidence of deterioration from a somewhat higher level of intellectual capacity was shown by the rather wide variations in the sub-test scores. The T score on the Hunt-Minnesota Test

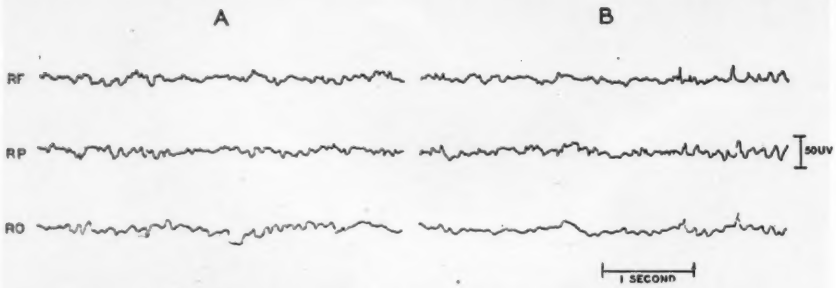

FIG. 3. Electroencephalogram after two weeks of treatment. (A—at rest; B—hyperventilation).

for Organic Brain Damage was 82. This value was well above the normal limits and signified that organic brain disease had affected intellectual functions.

TREATMENT

The patient was treated solely with oral Vitamin D and $CaCl_2$ (as the 25% solution). There were no dietary restrictions. The details of dosage and response of the serum calcium and phosphorus levels are shown in Figure 4. Clinically there was complete relief of symptoms after ten days on this regime. The signs of nervous hyperexcitability could no longer be elicited. The deep reflexes were present but were very sluggish. The spinal fluid pressure on Feb. 9, 1945, was 190 mm. H_2O. The patient was discharged from the hospital on Feb. 10, 1945, on a maintenance dose of 200,000 units of Vitamin D and 4 grams of $CaCl_2$ daily.

SUBSEQUENT EXAMINATIONS

The patient returned on March 12, 1945. She had been completely symptom-free since discharge from the hospital. The family commented on the remarkable improvement in her personality reactions and her ability to carry out a full day's activity without complaint.

The physical findings were essentially as on discharge. The slight changes in the eyegrounds were no longer evident. The serum calcium was 9.5 mg. per 100 cc.; the serum inorganic phosphorus was 5.7 mg. per 100 cc.; the serum alkaline phosphatase was 2.7 Bodansky units. Roentgenograms of the lumbo-sacral spine showed an increased density of the bony structures (no films of this area had been taken on the original admission). There was also a spina bifida involving the first sacral segment.

Intelligence tests were again given but in order to avoid artificially high scores due to practice effect the Stanford-Binet and Wechsler-Bellevue Scales were not used. On the

Shipley-Hartford Scale the patient scored a full I.Q. of 72 (Verbal I.Q.—66, Abstract I.Q.—78). A valid CQ could not be computed due to the low vocabulary score. The I.Q. as measured by Buck's Time Appreciation Test (3) was 70.

An EEG tracing on this date was almost within normal limits (Figure 5A). Some fast

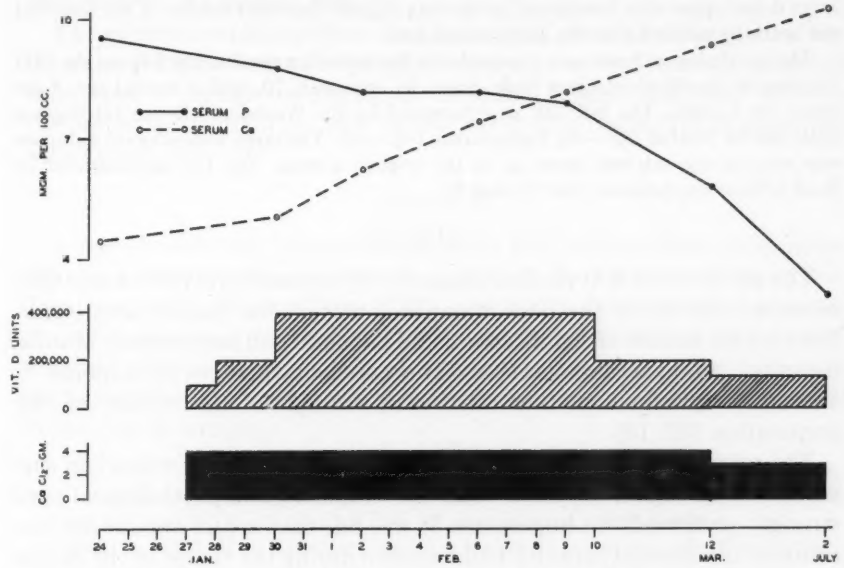

FIG. 4. Treatment schedule and response of serum calcium and phosphorus.

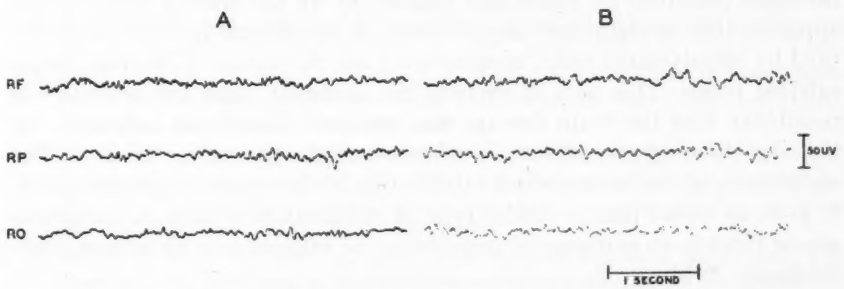

FIG. 5. Electroencephalogram after six weeks of treatment (A—at rest; B—hyperventilation).

activity was still present in the frontal areas. Hyperventilation (Figure 5B) no longer brought out the slow irregular activity so prominent in the first tracing but a few abnormal waves did appear. The daily dosage of Vitamin D and $CaCl_2$ was reduced to 150,000 units and three grams respectively.

The patient was seen again on July 2, 1945. The clinical improvement, on the whole,

was well maintained. There had been occasional episodes of weakness, largely subjective, which did not interfere with her activity. Her emotional adjustment had continued to improve. There were no changes of any significance on the physical examination. The serum calcium was 10.2 mg. per 100 cc.; the serum inorganic phosphorus was 3.1 mg. per 100 cc.; the serum alkaline phosphatase was 3.8 Bodansky units. Roentgenograms of the lower dorsal spine were interpreted as showing slightly increased density of the bone but not nearly as marked as in the lumbo-sacral area.

The psychological tests were repeated with the following results. The I.Q. on the 1937 Revision of the Stanford-Binet Scale, Form M, was again 70, with a mental age of ten years, six months. The full I.Q. as determined by the Wechsler-Bellevue Intelligence Scale was 82 (Verbal I.Q.—80, Performance I.Q.—88). The same wide range of variation was seen on the sub-test scores as in the original testing. The I.Q. as measured by Buck's Time Appreciation Test (3) was 72.

COMMENT

The present case is typical of idiopathic hypoparathyroidism except that increased density of the bone was noted only in the lumbo-sacral spine. No etiologic factors of significance could be found and intercurrent Monilia infection (14) was not observed. The excellent therapeutic response to Vitamin D provides another convincing example of the efficacy of this preparation (12, 13).

The psychiatric examination revealed impairment of the sensorium suggestive of an organic type of brain involvement. The psychological tests strongly confirmed this impression. It was felt that actual organic deterioration of intellectual capacity had occurred during the course of the illness, as was noted in another reported case (2). Treatment of the parathyroid insufficiency was thought to have improved the mental capacity in one of the cases described by Eaton and Haines (6). In the present instance it is apparent that no significant improvement in intellectual function, as measured by standardized tests, accompanied the restoration of normal serum calcium levels. This lack of reversibility however, does not obviate the possibility that the brain damage was initiated, directly or indirectly, by the long-standing abnormality of calcium and phosphorus metabolism. The significance of the intracerebral calcification in this regard is problematical. It is to be noted that a similar type of calcification is seen in conditions where there is no evidence of disturbance of calcium and phosphorus metabolism.

The spinal fluid pressure of 240 mm. H_2O was observed on two occasions, both with good relaxation. As in the remarkable case reported by Barr and associates (2), treatment resulted in a subsidence of the increased intracranial pressure. In their patient the total calcium and ionic calcium of the spinal fluid were at normal levels both before and after treatment. On this basis the suggestion was made that differences in concentration of calcium on the two sides of the cerebral membranes might have been con-

tributory to accummulation of fluid during tetany and to its disappearance after treatment. They also point out that edematous changes in the cerebrum or meninges may contribute to the cerebral and psychic manifestations of tetany. Unfortunately no determination of the spinal fluid calcium was made in the present case.

The electroencephalographic findings closely paralleled the clinical and laboratory improvement. A complete return to normal was not achieved, since even in the last tracing hyperventilation brought out slight traces of the previous abnormal pattern. In this respect the results are in agreement with Odoriz *et al* (11).

SUMMARY

A case of idiopathic hypoparathyroidism with intracerebral calcification, increased intracranial pressure, and mental deterioration is described. Treatment with Vitamin D and $CaCl_2$ resulted in complete relief of symptoms. Standardized psychological tests, given before and after recovery, revealed that no improvement of intellectual function accompanied the clinical response to treatment. Some degree of organic brain damage was thought to be present.

ACKNOWLEDGMENTS

I am indebted to Dr. David C. Wilson for permission to report this case.

I wish to thank Miss Rosemary Dew for technical assistance with the electroencephalograms and Mrs. Dorota Rymarkiewiczowa for the psychological tests.

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THE EXCRETION OF NEUTRAL 17-KETOSTEROIDS IN TWO CASES OF MALE PSEUDOHERMAPHRODITISM

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THE term, male pseudohermaphroditism, is ordinarily used to designate individuals whose gonads are testes and in whom some of the female organs of reproduction are present. The two cases reported in this paper fall in this class. Conversely, female pseudohermaphroditism refers to the condition in which the gonads are ovaries and some of the male organs of reproduction are present. In female pseudohermaphroditism it seems probable that the development of the clitoris into a short hypospadic penis and the formation of a male type of perineum is brought about by excessive androgenic activity of the fetal adrenals. There is no absolute proof of this, obviously, but the known hyperactivity of the adrenals following birth in four cases which have been studied by W. M. Allen (2) makes this explanation plausible. The hormonal relations in the male pseudohermaphrodite, on the other hand, which might explain the abnormalities of development are not so evident. [For detailed discussion see Young (11).] The excretion of 17-ketosteroids in the two cases of male pseudohermaphroditism to be reported herein seem to indicate that in early adult life there is no excessive production of androgens.

Case 1.

The patient was a colored girl, 15 years of age, first seen in the Homer G. Phillips Hospital, of St. Louis, in 1940. She was admitted to the hospital because of pain and swelling in the left groin, first noticed a few days previously after being hit in the pubic region while playing basketball.

The history prior to the injury indicated that she had developed normally, and was considered by her mother to be a perfectly normal girl except that menstruation had not as yet appeared. The development of the breasts and the appearance of pubic and axillary hair had apparently occurred in the twelfth and thirteenth years. Her school work was good and she was considered to be a well adjusted girl. Her interests were strictly of the feminine type. She stated that she was attracted to members of the opposite sex and admitted having had intercourse frequently although without much gratification.

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The physical examination showed a healthy appearing colored girl, five feet, four inches in height, who had a typically feminine habitus. The breasts were well developed, and the pubic, axillary and vulval hair were abundant, but there was no hirsutism. The clitoris was not enlarged, and the labia appeared entirely normal. The hymen was ruptured and the vagina was of normal size and depth. Rugae were well developed and the epithelium was moderately well stimulated. No cervix, uterus or ovaries were palpable. There was a firm, thin, transverse band extending laterally from both sides of the uppermost part of the vagina. A globular mass, similar grossly in every respect to a testicle, was present in the upper part of each labium majus, just outside the external inguinal ring.

The patient was considered to be a male pseudohermaphrodite, but no surgical interference seemed advisable; consequently she was discharged from the hospital and advised to continue her already well adjusted life as a girl.

The patient was next seen in the Barnes Hospital of St. Louis in 1943, again with a complaint of soreness in the left inguinal region. The examination at this time was essentially the same as in 1940. At this time the inguinal canal was explored, and the left testis and epididymis were removed. The structures about the gonad were hemorrhagic, probably as a result of trauma. The peritoneum was opened and the pelvic structures were explored. No uterus tubes, ovaries, prostate or seminal vesicles were palpable. A fibrous cord did course down to the vagina from each inguinal ring.

Microscopic sections through the testis showed well developed tubules and interstitial cells, but there was no evidence whatsoever of any spermatogenesis. The framework of sertoli cells was well developed. The epididymis seemed normal except for the absence of spermatozoa.

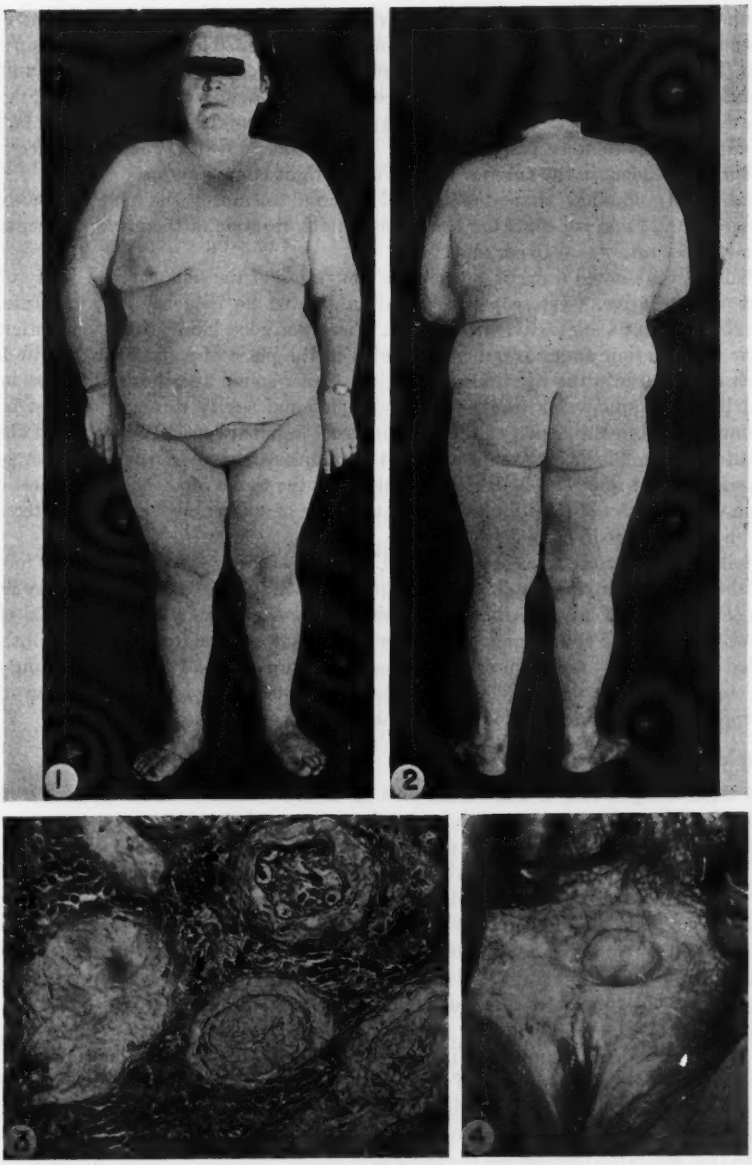
Case 2.

A 26 year old white female was admitted to the Ellis Fischel State Cancer Hospital at Columbia, Missouri, complaining of never having menstruated, of being obese for 15 years, and of intermittent swelling of the right labium majus for two years.

At the age of twelve the patient first noticed enlargement of the clitoris and breasts, the presence of pubic hair, and a deepening of the voice. There had been a large increase in weight, until at the age of 23 she weighed 110 kg. (242 pounds). On the advice of her physician she dieted for two years, reducing to 63.6 kg. (140 pounds). There had been a moderate amount of soft, fine facial hair for about eight years necessitating shaving the upper lip every three months. Two years prior to admission the patient first noticed a painless swelling of the right labium majus, and this persisted during the day but receded at night. The swelling, sometimes accompanied by epigastric discomfort, occurred at intervals of from three to six weeks until the time of admission.

The family history revealed that the patient's father, mother, two brothers and one sister were all sexually, physically and mentally normal. The sister is now twelve years old and has already begun to menstruate. The patient is a college graduate and seems to be well adjusted. As a child and young girl she indulged in the usual social activities. She has never had any sexual desires, and her few relationships with males have always been platonic.

Physical examination showed an obese (81.8 Kg., or, 180 lb.), short (19.44 cm., or 4 ft. 9 in.), alert and co-operative female (Fig. 1). The blood pressure was 135/80 mm. Hg. The skin was somewhat moist and flabby over the body folds. There was a moderate amount of soft, fine hair on the face. The larynx was not prominent and the thyroid was not enlarged. The breasts were atrophic (such as might be found in an obese male)



FIGS. 1–2. Patient at time of admission.
FIG. 3. Photomicrograph (low power) of biopsy of testicle. Note interstitial cell hyperplasia and atrophied tubules.
FIG. 4. Note markedly hypertrophied clitoris.

and the nipples and areolae small. The heart and lungs were normal. The abdomen was extremely obese and no organs or masses could be palpated. The pubic hair was fine and silky. The clitoris measured 2 cm. in diameter and 5 cm. in length (Fig. 2), and the urethral orifice was just above the vaginal orifice. The vagina was of normal depth, but admitted only one finger and nothing suggestive of a cervix or uterus could be felt. No adnexal masses were palpable. There was a firm, movable, slightly tender mass measuring 2×3 cm. in the anterior portion of the right labium majus.

Laboratory findings showed a normal glucose tolerance; the non-protein blood nitrogen was 23 mg. per cent; the Aschheim-Zondek reaction of the urine was negative; the metabolic rate was +15 per cent.

Skull plates showed a slight but definite elongation of the sella turcica. X-ray of the chest was negative. Cystoscopic examination showed no evidence of a prostate, although there was a suggestive median bar. There was good kidney function bilaterally. Pelvic examination under anesthesia revealed in the place of a fundus a fibrotic band which extended into the right adnexal region. Under spinal anesthesia the mass in the right labium majus was retracted into the abdominal cavity through a defect in the abdominal wall in the region of the right internal inguinal ring. A gonad could also be palpated in the left labium majus. A right hernioplasty was performed and the gonad biopsied but not removed. Microscopic sections of the tissue removed showed testicular tissue with atrophy of the tubules with no evidence of spermatogenesis. The interstitial cells, however, were hyperplastic (Fig. 3).

Following discharge from the hospital 1.0 mg. of stilbestrol was given daily for four months, but this produced nausea. Consequently 2.50 mg. daily of sodium estrone sulfate (*Premarin*) was given. After taking this dose for six weeks moderate vaginal bleeding was produced. Pelvic examination after the use of estrogen for about six months revealed a moderately well stimulated vaginal epithelium, a small cervix containing clear mucous, and a small uterus. The breasts likewise showed some development. The organs of reproduction, therefore, responded normally to the estrogenic hormone.

Steroid excretion

A 24 hour specimen of urine from each of these two cases was studied for steroids. The urine was collected in bottles containing 400 cc. of butyl alcohol, and as soon as possible was extracted three times with one-third volume of butyl alcohol. In both cases the 17-ketosteroids and the steroids conjugated with glucuronic acid were determined. The ketosteroid was determined with meta-dinitro benzene after hydrolysis in aqueous solution of a portion of the butyl alcohol extracts, using the method of Holtorff and Koch (8). The steroid conjugated with glucuronic acid was determined by the method of Allen and Viergiver (1) used for the determination of sodium pregnanediol glucuronidate. Whether or not this latter method serves to measure the ketosteroids which are normally conjugated with glucuronic acid remains uncertain. The fact is, however, that in one case of adrenal tumor and in four cases of female pseudohermaphroditism with adrenal hyperplasia the amount of steroid conjugated with glucuronic acid was significantly increased above that found in normal people. Furthermore, the amount of steroid determined in this fraction (by measurement of the glucuronic acid in the fraction) correlated well with the amount of conjugated ketosteroid measured by the meta-dinitro benzene method in the same fraction. In no case so far studied by the two methods (Allen, W. M.) has there been a large amount of 17-ketosteroid without there being simultaneously a corresponding increase in the amount of conjugated steroid measurable by the titremetric method for the determination of sodium pregnanediol glucuroni-

date. In one other case of adrenal tumor, however, the steroid glucuronidate was considerably increased above the normal, even though the 17-ketosteroids were only slightly above the normal. It would appear, therefore, that the utilization of the two methods lends added support to the conclusion that there was not an increase in the excretion of steroids in these two cases.

The 17-ketosteroids in the second case were 9.0 mg. per 24 hours, but the conjugated steroid was zero. This low value for conjugated steroid may have been due to hydrolysis prior to determination. In the first case the conjugated steroids were 11.5 mg. (equivalent to approximately 6.0 mg. of ketosteroids, and the 17-ketosteroids were 10 mg. per 24 hours). These results indicate that there was no excessive excretion of steroid material in either case. The values obtained are about the same as those obtained in this laboratory for normal women by the methods used.

DISCUSSION

The problem of diagnosis in *Case 2*, prior to biopsy of the gonad, presented some interesting possibilities. There were the obvious, gross developmental abnormalities, the short stature, the obesity and the large head. The childhood development was apparently normal prior to the age of twelve. Then the secondary sex characteristics began to appear. The pubic, vulval and axillary hair appeared but the breasts failed to develop and menstruation did not appear. In addition, the axial skeleton stopped growing, and marked obesity appeared; she had tall, lean parents and a normal sister and two normal brothers. All of this pointed to the first appearance of the abnormality at about the time of puberty. In many respects the cessation of growth resembled the stunting of growth such as is seen in precocious puberty, yet obestiy is not a characteristic manifestation of precocious gonadal development. The absence of hypertension and marked hirsutism seemed to make a diagnosis of adrenal tumor unlikely, and the general appearance seemed to rule out the Cushing syndrome. The most likely diagnosis seemed to be pseudohermaphroditism, chiefly because of the hypertrophied clitoris and the relatively rudimentary vagina.

The next problem was to determine, if possible, whether the condition was one of male or female pseudohermaphroditism. The biopsy of the gonad showed testicular tissue. Consequently the urinary steroids were studied to find out whether or not the amount excreted was normal, as in *Case 1*, or elevated as in female pseudohermpahroditism. In four female pseudohermaphrodites (aged 4, 7, 21 and 27 years) which one of us has studied (Allen) there has been in each a considerable increase above the normal in the amount of 17-ketosteroid and total conjugated steroid (measured by the method for determining sodium pregnanediol glucuronidate). In these cases the amount excreted has increased as the subjects have progressed to adult life. [See also Talbot *et al.*, (10), Fraser *et al.*, (5); Engstrom *et al.*, (4).]

Emphasis should be placed on the fact that abnormalities in pseudohermaphroditism of either the male or female type are present at birth although the subsequent development may further depart from the normal because of subsequent hormonal influences. This is especially the case in the four female pseudohermaphrodites mentioned above. The younger two, aged four and seven, both showed pubic and axillary hair when first seen, and in the youngest pubic and axillary hair appeared in the first year after birth. Furthermore, the amount of ketosteroid excreted was greatest in the two mature individuals, indicating that the adrenals produce increasing amounts of androgen as age increases. The masculinizing effects of the androgen are manifested by amenorrhea, a low pitched voice, a masculine torso and moderate hirsutism. If there were no continuation after birth of the masculinizing influences, these genetic females should develop into normal females except for the developmental defects present at birth. In the female pseudohermaphrodite the primary cause would appear to be hyperplasia of the adrenal cortex. Furthermore, it must be assumed to begin sometime prior to birth, but not too early, for if it occurred very early the reproductive organs would probably not be so normally developed. (The main abnormalities are a virtually closed vagina and hypertrophy of the clitoris.)

The origin of the developmental defect in the male pseudohermaphrodite is more complex. Male pseudohermaphroditism shows many gradations from the moderately masculine type illustrated by *Case 2*, to the predominantly feminine type seen in *Case 1*. Since they are both assumed to be genetic males, the feminizing effect appears to be considerably stronger in the first case. The vagina and external genitalia were of the normal female type, the clitoris was not enlarged and there was no hirsutism. It must also be assumed that the testes (or possibly the adrenals) were able to produce estrogen beginning at the proper time during adolescence. Otherwise the breasts would not have developed normally and the body would not have been of the female type. In the second case, however, there was a tiny uterus and a small vagina, and, some hirsutism.

In each of these cases of male pseudohermaphroditism there was no increase in the excretion of ketosteroids or total steroid. They are distinctly different from the female hermaphrodites, therefore, in their endocrine makeup following birth. Furthermore the pubic and axillary hair did not appear until adolescence. This suggests that the testes begin to function at the normal time, although in these two cases spermatogenesis was absent.

The cause for the development of the female organs of reproduction in a genetic male, in so far as actual proof is concerned, in the human being is unknown. In some cases there is a definite hereditary basis. A compilation of some reported cases shows the sex ratio to be one to one, only if the

pseudohermaphrodites are considered as genetic males [Witschi (11)]. The females are apparently normal but in some instances the normal appearing males are sterile. The intersexes, if they marry as females, as they occasionally do, are obviously sterile.

In the two cases recorded here the sex ratio more closely approaches the normal if they are considered as genetic females. The total number of brothers among the siblings is six whereas the sisters are only three. If the two pseudohermaphrodites are considered as genetic females the ratio is six boys to five girls. This cannot, however, be used as a very strong argument in favor of the consideration of them as genetic females since the gonads are so obviously testes. The argument does not need to be a closed one, however, because in the free-martin in cattle [Lillie (9); Chapin (3)] the female twin of a male in which there is fusion of the chorions may actually be so masculinized that the gonad which began life as an ovary resembles both grossly and microscopically the sterile testes seen in these two patients.

The assumption that the cause is genetic still fails to explain the defective development. In the light of Greene's studies (6, 7) on the production of hermaphroditism in unborn rats by the administration of sex hormones to pregnant rats it becomes much easier to understand what may happen. He has shown that the administration of an androgen to pregnant rats causes the females to be born showing considerable masculinization. The clitoris is hypertrophied, the vagina fails to canalize normally and the male accessories are stimulated. Conversely, when an estrogen is given the males in the litter are feminized. These extraordinary findings change the whole interpretation of pseudohermaphroditism. In these two cases, if they be genetic males, it seems probable that excessive amounts of estrogen were produced at a critical period of development so that the female structures which normally fail to develop in the male were stimulated while the structures normally developing in the male were inhibited. The source of the excess estrogen remains unknown. It may come from the mother's ovary, from the placenta or even from the fetal gonads or adrenals themselves. If the mother's ovary were at fault one would expect hermaphroditism of this type to be much more frequent than it is.

If these cases be considered as genetic females, it seems probable that a sufficiently strong masculinizing influence was present during embryonic life to completely suppress the development of the cortex of the primitive gonad and thus permit the medulla to develop into a sterile testis.

SUMMARY

Two cases of male pseudohermaphroditism are described. In each the excretion of 17-ketosteroids was normal. In one case the uterus was absent,

but the external genitalia, vagina and breasts were of the normal female type; whereas, in the other, the clitoris was enlarged and the vagina, uterus and breasts were atrophic. In the latter case uterine bleeding was produced by the use of estrogens.

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Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: D. A. McGINTY. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, I. BRAM, F. A. DE LA BALZE, ANNE P. FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MACARTHUR, T. H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZO-MEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH AND L. T. SAMUELS.

GONADS

BANCROFT, P. M. Inguinal ectopia of the ovary. *J. Pediat. 26* (5): 489–492 (1945).

The cases of two infants of two months of age are reported. The first had an inguinal ovary and tube that became strangulated and gangrenous after attempts at reduction and had to be removed. The second had an inguinal hernia into which a normal ovary and tube prolapsed; the organs were readily reduced and the ring closed surgically. The author uses the classification of Donald for ovaries in the inguinal canal: 1) inguinal hernia of the ovary, where intra-abdominal pressure has forced the ovary into a patent canal; and 2) inguinal ectopia of the ovary, where the ovary is pulled into the canal by shortening of the gubernaculum or by failure of the medial attachment. The author concludes: 1) the majority of cases of inguinal ectopia of the ovary occur in the first two years of life; 2) its incidence is probably greater than is indicated by the literature; 3) the diagnosis is, as a rule, not difficult if the anomaly is found before torsion or trauma have altered the findings; 4) examination of the female genitalia in infancy should be made with the same care as is the rule in the examination of the male; and 5) early surgical intervention with restoration of the abdominal viscera and repair of the patent inguinal ring is the treatment of choice.—*E.C.R.,Jr.*

MASSON, G. Spermatogenic activity of various steroids. *Am. J. M. Sc. 209* (3): 324–327 (1945).

Immature male albino rats were hypophysectomized and then given two injections daily of one of 16 steroids for ten days at which time the animals were sacrificed, the testes were weighed and the presence of spermatozoa in the seminiferous tubules was determined. All steroids were given in the dose of 2 mg. per day. As controls, one group of animals was non-operated and sacrificed at the beginning of the experiment; a second group was not operated and kept for a period of ten days; and a third group was hypophysectomized and treated for ten days with cholesterol (a pharmacologically inert substance). The compounds with the most marked spermatogenic activity were androstenediol dipropionate, methyl androstenediol, Δ^5-pregnenolone (this compound was not active in a 1 mg. dose), methyl androstanediol, androstenediol, and dehydroiso-

androsterone. Other compounds, including methyl testosterone, testosterone, progesterone and androstenedione, partially protected the testis from atrophy but had no spermatogenic activity in the dosage or the time interval studied. Ethinyl testosterone, desoxycorticosterone acetate, and acetoxypregnenolone were practically inert. The author emphasizes that: 1) none of the steroids so far studied is able to promote a normal growth of the gonads in the absence of the pituitary; 2) no relationship necessarily exists between the weight of the testis and the activity of the germinal epithelium, and 3) the ability of steroids to protect the testis against the atrophy caused by hypophysectomy is entirely independent of the other main pharmacologic activities.—*E.C.R., Jr.*

MILLETT, J. AND J. SHELL. Meigs' syndrome in a case of multilocular pseudomucinous cystadenoma of the ovary. *Am. J. M. Sc. 209* (3): 327–335 (1945).

Meigs' syndrome (hydroperitoneum and hydrothorax with a benign pelvic tumor) was originally described in association with a fibroma of the ovary. The authors have reviewed the literature and with the concurrence of Meigs have broadened the syndrome to include other benign pelvic tumors. They report a case with recovery due to a massive multilocular cystadenoma of the ovary; this is the second case of this type to be noted in the literature. The authors point out that the recent increase in reported cases is probably due to increased recognition of the condition rather than to an increase in incidence. They emphasize that both internists and surgeons should be made more aware of this condition as the outcome of the undiagnosed case is fatal, as compared to the 100 per cent recovery in the properly treated case.—*E.C.R., Jr.*

QUINBY, W. C. Undescended testicle. *New England J. Med. 232* (24): 701–704 (1945).

After discussing the physiology of the testis the author comes to the conclusion that a testis which has not descended at puberty should be given immediate treatment. Endocrine treatment should be tried first in all cases because, even if it is successful in only a few cases, it causes the growth of the parts which makes the subsequent operative procedure easier.—*L.T.S.*

HYPOPHYSIS

GROSS, M. The relation of the pituitary gland to some symptoms of alcoholic intoxication and chronic alcoholism. *Quart. J. Stud. on Alcohol 6:* 25 (1945).

On the basis of extensive review of the literature it is suggested that many unexplained symptoms of alcoholic intoxication and chronic alcoholism may be related to disturbance of the hormonal secretions of the pituitary gland. While acute effects of alcohol are due to its narcotic action, most diseases of chronic alcoholism have been ascribed to concomitant subvitaminosis. It seems reasonable to relate the unexplained manifestations of chronic alcoholism and symptoms of acute intoxication and the posttoxic state whether to direct chronic effects of alcohol itself or to the effect of concomitant subvitaminosis on some organ particularly susceptible to the action of alcohol or subvitaminosis. The implicated organ would have to be sufficiently important in the functioning of the whole organism to be capable of influencing a wide range of functions disturbed in chronic alcoholism. This organ would also probably be composed of a large amount of nervous elements, since both the narcotics and subvitaminotic effects of alcohol have been shown to be manifested in the nervous tissue. The organ which meets

these requirements is the pituitary gland. Histological findings reported in the literature support the hypothesis that the pituitary gland is affected in chronic alcoholism and thus may be responsible for some of the symptomatology of this condition, including fatty liver and gastritis.—*Courtesy Biol. Absts.*

WAINE, H., G. A. BENNETT, AND W. BAUER. Joint disease associated with acromegaly. *Am. J. M. Sc. 209* (5): 671–687 (1945).

The authors report the case summary and complete postmortem findings of a 58 year old man with acromegaly of 20 years duration. A detailed pathologic study of the joint lesions is reported. It was found that in many respects, particularly where there were advanced alterations, the histologic picture closely resembled that of severe degenerative joint disease. In some features and in a few individual sites, however, the changes appeared to be of a wholly different nature and could not be regarded as reactions to primary degeneration of cartilage. These phenomena, principally new growths of cartilage and bone, were entirely consistent with the lesions originally described as characteristic of acromegaly by Erdheim (Virchows Arch. f. path. Anat. 281: 197, 1931). Briefly they included: 1) additional growth of the vertebral bodies and the intervertebral disks of the spine; 2) hyperplasia and hypertrophy of the columnar zone of chondrocytes associated with an increase in the matrix in the joint of one toe; and 3) nodal and circumferential growth in the costochondral cartilages. The authors conclude that these phenomena appear to indicate reactivation of cartilage growth and enhanced endochondral ossification, unphysiologic at the age of this patient and perhaps the result of a specific hormonal stimulus.—*E.C.R., Jr.*

PANCREAS

BARACH, J. H. Normal standards in the treatment of young persons with diabetes. *Am. J. Dis. Child. 69:* (2) 92–98 (1945).

The author has gathered from the literature and averaged the age-height-weight tables of more than 400,837 boys and girls in order to portray the normal trends of growth from the ages of one to 21 years. Similarly a table of diets was constructed after a survey of the generally accepted values for caloric requirements per day and daily protein requirements per kilogram of body weight of growing children and of the amounts considered normal and adequate for children of various ages. The growth curves of 135 diabetic children under observation for two to 12 years were compared with the normal age-height-weight charts. By these standards, 25 of the diabetic children were classed as normal, and were less than two inches above or below the ideal height, and less than five pounds above or below the ideal weight; 57 were classed as undersized, and of these 31 were more than ten per cent below the normal height and 31 were more than ten per cent below the normal weight; and 53 were classed as oversized, of whom 39 were more than ten per cent above the normal height and 15 were more than ten per cent above the normal weight. A study of the caloric intake of these children revealed that nine of the normal children achieved normal growth in spite of subminimal diets, and 16 showed normal growth in spite of excessive diets. Of the undersized children, 30 received inadequate diets, and none maximal or excessive diets; of the oversized children, 22 received excessive diets, and none minimal or inadequate diets. The generously fed children required 1.5 to nearly 2 times as much insulin as those who had been subsisting on conservative diets. The author concludes that there is a positive relationship between the caloric value of the diet and the growth of the diabetic child.—*E.C.R., Jr.*

BIASOTTI, A., V. DEULOFEU, J. R. MENDIVE AND A. PATALANO. Insulin-zinc-histone in the treatment of diabetes. *Re. Inst. bact. 12:* 227 (1944).

Insulin-zinc-histone, insulin-zinc protamine, and crystallized insulin were compared. A group of diabetics, ranging in age from eight to 64 years who required insulin for maintenance were found to progress satisfactorily in regard to carbohydrate metabolism, weight, growth, and ability to work, with absence of severe complications, upon the administration of insulin-zinc-histone. The rapidity of its action and the prolongation of its effects were found to be intermediate between that of insulin-zinc-protamine and crystalized insulin. The action of this preparation appears to depend upon the presence of free insulin. The use of histone is also advantageous since, at this time, protamine must be imported in Argentina.—*Courtesy Biol. Absts.*

DREY, N. W. The clinical interpretation of insulin induced ketonuria. *Ann. Int. Med. 22:* 811–818 (1945).

Four cases of insulin induced hypoglycemia followed by the appearance of ketonuria are presented. Two of the patients had diabetes mellitus, one had renal glycosuria and one neurogenic hypoglycemia. Because of failure to appreciate the fact that insulin may be either anti-ketogenic or ketogenic, the clinician may sometimes administer additional insulin when acetone appears when in reality no insulin or more carbohydrate or both are indicated. Following the administration of too much insulin, there is an excessive outpouring of glycogen from the liver in an attempt to correct the hypoglycemic state. During this phase, fat metabolism is stepped up and the ketonemic level increases.—*J.M.*

DUFF, G. L. The pathology of the pancreas in experimental diabetes mellitus. *Am. J. M. Sc. 210* (3): 381–397 (1945).

The author summarizes the essential observations in this field; the paper contains 64 references. He compares surgical diabetes produced by partial pancreatectomy, endocrine diabetes produced by anterior pituitary extracts, chemical diabetes produced by alloxan, and human diabetes mellitus from the pathological point of view, and concludes that none of the experimental conditions successfully reproduces the lesions of the human disease. The possible role of surgical, endocrine, chemical and other factors in the etiology of human diabetes mellitus is considered.—*E.C.R., Jr.*

LOWRY, P. T. AND D. M. HEGSTED. The thiamine requirement in alloxan diabetes. *J. Lab. & Clin. Med. 30* (10): 839–843 (1945).

A measured diet consisting of 74 per cent sucrose, 18 per cent casein and 4 per cent fat was fed to intact rats in which thiamine deficiency was produced and to two groups of animals made diabetic with alloxan, in one of which a thiamine deficiency was simultaneously brought about. Changes in body weight in relation to the withholding or feeding of thiamine were used as criteria for recording absence or presence and degree of thiamine deficiency. The tendency of the diabetic animals to develop thiamine deficiency was "approximately the same or perhaps slightly less than the controls." Therefore, the authors feel that their work does not support the theory "that diabetic neuritis is due to thiamine deficiency." However, they caution against accepting the presented facts as conclusive proof that diabetes in the human being can be compared to the diabetes of rats produced by alloxan.—*T.H.McG.*